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Seven questions for the energy secretary

BRITAIN now has a Department of Energy whose Secretary of State is Lord Carrington, previously Secretary of State for Defence. The brief of this new department is, in the words of the Prime Minister, "to concentrate on the development of the coal industry on nuclear power and on our offshore oil and gas resources at home, as well as on those tasks of working together with other oil-consuming countries and with the oil-producing countries on the international aspects of the energy problem".

The following energy issues will ultimately deserve his or his successor's attention.

How permanent is the department to be? It is unlikely that the problem of finding adequate, reasonably priced energy sources is going to go away before the turn of the century. Many scientists and technologists could become enthused by the thought of a long term commitment to a strong government department pursuing such desirable objectives as rethinking energy strategy. They could not face, however, being pushed around from ministry to ministry each time the Cabinet was reshuffled.

Can energy be removed from the political arena? There are many aspects of energy policy which merit serious discussion but the subject can easily become a political football. The inferior quality of the recent Commons debate on the fuel shortage does not bode well for informed non-partisan discussion in the future. Lord Carrington is, of course, a political creature holding simultaneously the post of Chairman of the Conservative Party. At the moment he is probably as obsessed with the thoughts of election strategies as with energy policy and in the long run it is desirable, assuming he wishes to remain as Secretary of State for Energy, that he does not divide his talents, particularly with such a politically exposed position.

Can some order be brought into research funding? Some weeks ago we reported on an energy strategy devised for the United States (246, 370; 1973). An important feature of it was that it was not intended to be competitive. Funding for coal was not to be at the expense, say, of funding for validating the nuclear option, nor was the coal industry in the process of its research expected to denigrate the nuclear industry. A strong impression left by many British pronouncements is that developments are largely made for this very purpose. It is therefore highly desirable that an urgent new look at funding mechanisms be made, in order to impose, if necessary, a degree of overlordship by an energy department.

Consequent on this, why not a think tank? Britain is not short of experts in almost all individual fields

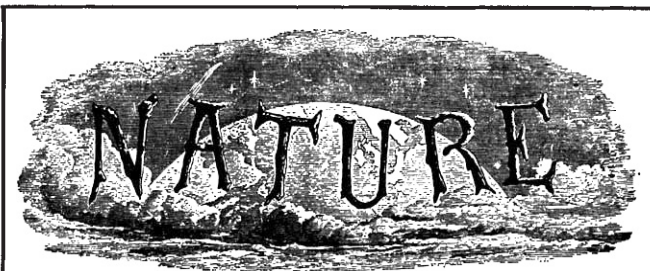
of energy research and use. It is, however, desperately lacking in people with wider vision. The reasons need not be repeated here. Yet without such people, and they should not necessarily be civil servants, an unreasonable load is placed on a minister in making decisions. A think tank would at least be a means of educating a wider range of people in strategic thinking.

Cannot conservation efforts be improved? Remarkably little has been done in the way of education for saving fuel and yet there are very attractive opportunities. Why does Lord Carrington not visit a power station and invite, in front of television cameras, all viewers to turn off something electrical? The drama of a meter needle moving under the influence of one's own action in the home would rival Mr Geller's activities.

How will Britain react to the Kissinger energy initiative? At the same time that it was being 'warmly welcomed', Britain and France were doing bilateral deals wherever possible—deals which, however desirable in the short term, cannot have a long term future within the spirit of international cooperation. However cynical one may be about the United States leading such an initiative while consuming energy so greedily and often wastefully, in the long run someone had to recognise that energy is so much a supranational and supra-oil company affair that it has to be dealt with internationally.

And, this being so, should Britain take steps to safeguard the interests of other countries, particularly developing ones, for whom increased energy prices are much more economically damaging than for industrialised countries? The early formulation of an international attitude to energy is possibly the most urgent need there is today. It will be of great interest to see whether the big energy consumers can see beyond their own industrial landscape.

100 years ago



Our country depends for its high position among nations, not only on its resources in coal and iron, but also, and more securely, on the mental capacity of its people, whose peculiarity is that they have the power of always using the resources at their disposal to better advantage than any others. We in nearly all cases have taken the lead in invention. A

From *Nature*, 9, 237, January 29, 1874.



Is population a ministerial responsibility?

Mr D. R. Cope of the Institute of Planning Studies, University of Nottingham, questions the need in Britain for a minister with special responsibility for population matters.

THE announcement by the Prime Minister, Mr Edward Heath, just before Christmas that Mr James Prior, Lord President of the Council, is to be minister with special responsibility for population matters means that one of the major recommendations of the Population Panel, set up in November 1971, has become government policy (*Report of the Population Panel*, HMSO; 1973). Population is by no means Mr Prior's only responsibility, as several pressure groups campaigning for a separate ministry were quick to point out, but his appointment raises the question of what are the areas of action open to a minister for population and what should be his priorities.

As the panel noted, quite a wide range of government activities, especially health, social welfare, fiscal and planning policies, may have an impact on population trends, but to date no government has formed policies with particular regard to the possible impacts. Is this situation likely to change?

The current demographic position in Britain is that no serious economic or social problems which could be directly attributed to either its population size or growth rate are likely to emerge in the next thirty or forty years. Present fertility behaviour will, of course, have an effect on population levels in the first decades of the next century and a minister of population will be concerned with what are, for economists and social planners, very long term futures. For politicians, such a long term concern is almost unprecedented and the potential unpalatability of a current policy designed to affect the demographic situation in the next century would be so great as to deter any government from incorporating an overt population policy in its programme of action.

Will the British government pronounce an opinion on an 'optimum' national population? The considered opinion of most academic demographers, such as Professor D. V. Glass of the London School of Economics, is to dismiss the concept as providing no useful base for policy making. Different criteria produce a congeries of optima, many of which conflict with each other. Like the legendary castle which always stayed on the horizon no matter how far or fast travellers rode towards it, an optimum population, if it could be defined, would be unobtainable. In the time taken to achieve a stated population, external circumstances would almost certainly have altered to give rise to another optimum.

The major field of potential action in population matters is in family planning policy. The present government has already incorporated an extended service in the health service reorganisation bill, though it was motivated as much by considerations of general social welfare as by any demographic consequences. It is well known that even if every birth in Britain were to be 'wanted'—if there were to be perfect use of contraceptive facilities—current family size patterns mean that the population would continue to grow. Demographically, a policy of freely available contraceptive facilities and a free abortion service to take care of cases of contraceptive failure or unavailability would have the greatest potential impact. Abortion can be a very effective agent in influencing population patterns but the sensitivity of the subject is again likely to deter governments from

too firm or blatant a policy relying on it.

There are really only two areas in which a population minister could identify immediate action needs: in demographic research and in publicity and information. Despite recent increases there is still far too little serious demographic research being carried out in Britain. Over the past few years many unsubstantiated assertions have been made on the relationships between population and the economy and environment, and about the impact of human numbers and densities on social and psychological well being. The need now is for research to test some of these interrelationships, especially factors affecting fertility behaviour. Demographers are also hopeful that Mr Prior's appointment will make it difficult for the government to cancel the proposed 1976 census on the grounds of economy, as a ten year intercensal gap is manifestly inadequate for determining changes in trends.

The Population Panel placed great emphasis on the need for government action on publicity and information about population trends and patterns. Obviously, a family planning service will be fully successful only if all those who could use it are aware of it and do use it. The panel, however, looked beyond such simple publicity and argued the necessity of bringing home to the public the need to consider the aggregate consequences of their family building behaviour, despite the possible conflict of these consequences with individuals' preferences. This reminds one of government attempts to do much the same in the context of income restraint and no doubt the public response would be very similar.



James Prior, Lord President of the Council

It will be interesting to see how long the position of minister with responsibility for population matters survives, given the very limited possibilities associated with it. Demographic trends may even rapidly make it superfluous if the position is seen as being specifically concerned with problems of population growth. As yet the post-1964 downturn in the numbers of live births shows no signs of reversing and despite allusions to the 'New York blackout syndrome' (which was a myth anyway, according to Udry, *Demography*, 7; 1970), current economic difficulties may well lead to an even greater decline in birth numbers with couples avoiding additional expenses by postponing births. Widespread advance warnings of economic rigours and the much greater use of the oral contraceptive pill mean that Mr Prior will not have to concern himself with a marked upturn in births in the last quarter of this year.

international news

Credibility gap worries US energy office

Colin Norman, Washington

After being deluged with dire warnings about the energy crisis that was supposed to hit the United States this winter, many Americans are now beginning to wonder whether there is a crisis at all, or whether the whole thing is an elaborate plot by the oil industry to drive up prices and profits. So far, there have generally been adequate supplies of gasoline and heating oil, cities are still brilliantly lit at night by hideous advertising signs, and life is proceeding more or less normally for most people. About the most tangible sign of energy shortages is that gasoline and heating oil have increased in price.

According to William Simon, Director of the Federal Energy Office, his biggest problem in the next few months is the growing public scepticism about the energy crisis. He is anxious because such scepticism could lead to backtracking on conservation measures which have so far turned out to be remarkably effective in curbing demand, and that in turn could precipitate a full-blown crisis before the summer arrives. And the problem of maintaining public support will become even more difficult once Arab oil again starts flowing into the United States. In fact, the Administration is so concerned about the matter that President Nixon made a radio address last weekend during which he warned that "if we permit ourselves to slack our efforts, to slide back to the wasteful consumption of energy, then the full force of the crisis will be brought home to Americans in a most devastating fashion, and there will be no longer any question in anyone's mind about the reality of the crisis".

The scepticism stems not only from the fact that there has been little discomfort so far in the United States, but also from a series of reports of record profits in the oil industry, rumours of tankers sitting outside ports waiting for oil prices to creep up before they unload their cargoes, and allegations that storage tanks at refineries are brimming with heating oil. But perhaps the most damaging blow to the credibility of the

crisis is the growing public awareness of the fact that the government has been forced to rely almost exclusively on the oil industry itself for statistics about production and stocks of oil. As Mr Silvio O. Conte, a Republican Congressman from Massachusetts, put it last week, that is like "putting a fox in to watch the chicken coop".

The Administration is thus beginning a campaign to assure the American public that the crisis is real, and that conservation measures are still necessary. First, President Nixon promised in his radio message that he will send a bill to Congress which would impose a windfall profits tax on oil companies to force them either to turn over excess profits to the government or to invest them in exploration for new sources of energy. And second, the Administration is sponsoring a bill which will, in Nixon's words, "require the oil companies to provide a full and constant accounting of their inventories, their production, their costs and their reserves". And, in the meantime, Simon has ordered a full audit to be conducted on the companies' figures. Those measures should, at least, help dispel some of the public distrust over the figures.

But, although the government has been relying on industry data for its analysis of the energy situation, there is still good reason to believe that the shortages are real, and are not solely the product of an inspired public relations exercise by the oil companies. As S. David Freeman, Director of the Ford Foundation Energy Policy Project, suggested last week, the companies have certainly been making a lot of money out of the situation, and the data they have supplied through the American Petroleum Institute may not tell the whole truth and nothing but the truth, but he suggests that they still indicate the need for gasoline rationing to be imposed in the spring.

And there is also good reason to believe the Administration's assertions that if the American public slackens in its conservation efforts, a severe crisis could still develop. For one thing, a variety of data supplied by the electricity industry, by the oil companies and by independent analyses all attest to the fact that conservation efforts have been remarkably successful in decreasing demand, even if the unusually warm weather in the last quarter of 1973 is taken into account. And for another, a

lot of oil has been flowing into the United States through the embargo, and this is now beginning to dry up—and even if the embargo is suddenly lifted, it will take several weeks for the oil to reach United States ports.

One of the most direct indicators of demand for energy is the amount of electricity supplied to consumers. According to data produced by the Edison Electric Institute, at the end of last year, consumption of electricity throughout the United States was averaging about 1.5% below that of a year previously. Moreover, during the week ending on January 12, demand dropped by 4.1%. Compared with an expected increase of about 7%, those figures show a very substantial decrease in demand.

In his radio address last weekend, President Nixon also cited a sheaf of statistics to show that demand for other forms of energy has declined equally dramatically. Consumption of gasoline in December was reduced by 9% below expected levels, for example, natural gas consumption was down by 6% compared with last year, and he also referred to a study in the North East which showed that some 19,000 households had reduced their heating oil consumption by an average of 16% compared with last year, even after adjustments had been made for the warmer weather.

The Administration still reckons however, that during the first three months of 1974, supplies of oil will fall short of previous demand by about 2.7 million barrels a day, or about 15%. This must be accounted for either by rationing or by conservation measures. Heating oil is already being allocated to parts of the country which are especially hard hit by the oil embargo (in other words, it is being rationed at the wholesale level), and last week the Federal Energy Office published details of a gasoline rationing plan which will be brought in if necessary, although no earlier than March.

In any case, President Nixon declared last weekend that he is opposed to gasoline rationing, and will avoid bringing it in if at all possible. He said, for example, "if this voluntary cooperation (in reducing consumption) continues, . . . we can avoid gas rationing this spring . . . and you can be sure that I will do everything in my power to achieve the goal of avoiding gas rationing."

Pollution research in a sorry state

Colin Norman, Washington

THE federal government's research and development programmes for cleaning up water pollution are starved of money, poorly managed and badly coordinated, according to a massive study carried out by the General Accounting Office (GAO). Unless the programmes receive more funds, the GAO said in a report made public last week, the goals set by the 1972 Water Pollution Control Act will not be met. Charges of financial undernourishment are commonplace in Washington, but in this instance they gain considerable credibility from the fact that the GAO, which is an investigative agency of Congress, has no axe to grind since it does not have a direct stake in the matter.

The Water Pollution Control Act, which was passed by Congress in December 1972, specifies that waterways in the United States should be clean enough to swim in by 1983, and that no pollutants at all should be discharged into navigable waters by 1985. Those are tough goals to meet, and they will clearly require massive investment of funds in sewage treatment plants. They will also require a vigorous programme of research and development on new control technologies.

Far from pursuing a vigorous programme, however, the Administration has cut some research budgets and failed to disseminate results from the studies which are being carried out. Moreover, the GAO charges that no overall strategy for research and development has been worked out by the Environmental Protection Agency, and the research is therefore not sufficiently geared towards meeting the goals specified in the Water Pollution Control Act.

Between July 1969 and June 1973, about \$495 million was spent by twelve federal departments and agencies on water pollution research, some \$238 million of which was spent by the Environmental Protection Agency (EPA). Although the EPA is supposed to be the focal point in the federal government for pollution control research and for enforcing federal environmental laws, its water pollution research budget was actually cut by the Administration in the 1973 fiscal year, from about \$50 million to \$42 million. Meanwhile, the water pollution research programmes of other agencies doubled between 1969 and 1972, increasing from \$36 million to \$71 million.

A prime objective of the EPA's research programme should be to minimise the costs of treating municipal sewage, because huge sums of money will be spent in the next few years on

treatment plants—the 1972 Act, for example, authorises expenditures of \$18,000 million between 1973 and 1975 for grants to help in the construction of sewage plants. A small percentage saving from research would clearly save large sums of money.

But the GAO report points out that although \$3,000 million was earmarked for municipal sewage grants in 1973, only \$9.5 million was earmarked for the development of control technologies. That translates to an investment of only 0.3% in research, and compares with a rate of 8% in the Department of Transportation's urban mass transportation programmes, and a rate of about 4% in industry, the GAO report notes.

A similar situation exists in another important EPA research programme—research aimed at determining how pollutants get into water, what happens to them, and what their effect is (so-called process and effects research). The GAO found that because of funding limitations and poor management, important research needed to establish water quality standards has been delayed, and research on thermal discharges has been inadequate. As far as the latter programme is concerned, the GAO report notes that thermal pollution is likely to be a rapidly growing problem as the number of power plants increases, but research is proceeding slowly, and according to the director of one EPA

laboratory, the agency has not even begun to solve the thermal pollution problems likely to be present in 20 years' time.

The GAO also found that there is a lack of coordination between the government's research and that conducted by industry, to such an extent that "we were informed (by representatives of industry) that industry was reluctant to reveal to EPA the level of technology developed to control pollution because (EPA) might speed up its enforcement action and industry might suffer a financial loss".

What needs to be done to alter this sorry state of affairs? Apart from increasing expenditures on research and development, the GAO report recommends that EPA should draw up a research and development strategy which would estimate the amount of money needed to meet the goals set out in the Water Pollution Control Act. The Office of Management and Budget should designate a federal agency as the focal point for coordinating research, and for disseminating research results, and ensure that EPA gets full cooperation from other agencies in drawing up its research and development strategy.

As for the Congress, the GAO suggests that since it is doubtful that waterways will be cleaned up by 1983 with present research funding levels, some thought should be given to increasing research appropriations. The

Sherman quits NIH

Colin Norman, Washington

A TOP official at the National Institutes of Health has resigned in frustration over the Administration's policies for biomedical research in general and for NIH in particular. Dr John Sherman, Deputy Director of NIH since 1968, and an NIH employee since 1953, will be leaving early in March to join the staff of the Association of American Medical Colleges, a Washington lobbying organisation.

Sherman said in a telephone interview last week that he has resigned "because of a number of tangible and intangible things" which are eroding the prestige of NIH and "making it a less attractive place to do research in". Chief among Sherman's complaints are the fact that the Administration has cut the number of personnel positions at NIH, and because he finds a lack of understanding between officials in the agency and administrators in the Department of Health, Education and Welfare.

As far as staffing is concerned, Sherman pointed out that personnel have been cut back at a time when the budget has been expanding, and when

greater emphasis is being placed on contract research as opposed to grants. Contracts take more time to administer, and therefore require more staff.

For some time, there has been general discontent at NIH over policies dictated by the Nixon Administration. Many of the complaints have revolved around budgetary problems, because the Administration cut many of the institutes' research budgets last year, and also because Congress and the Administration failed to agree on a budget for the agency for almost 18 months. But Sherman's complaints go deeper than simple lack of money. "There is a sort of a sense of negativity (in the Department of Health, Education and Welfare), and a lack of understanding between us and the decision-makers", he said, and many of the frustrations stem from the strict management control that the Administration is trying to place on basic biomedical research.

Sherman first joined NIH in 1953, he has been an Associate Director of NIH for Extramural Programs, and was appointed Deputy Director in November, 1968. When Dr Robert Q. Marston resigned as NIH Director a year ago, Sherman was appointed acting director for some six months.

problem is, however, that the Administration needs to be persuaded to spend money for water pollution control which has been earmarked by Congress. So far, the Office of Management and Budget has released less than half of the money for sewage treatment grants which Congress authorised in the 1972 Water Pollution Control Act.

New cyclotron for neutron therapy

John Hall

BECAUSE of Hammersmith Hospital's success in the use of neutron therapy for the treatment of cancer a second British hospital is to be supplied with a compact cyclotron. The Medical Research Council (MRC), the Scottish Home Office and Health Department, and the Cancer Research Campaign will jointly finance a unit costing £819,000 at the Western General Hospital Edinburgh, which should be ready to treat patients by the summer of 1976. Hammersmith Hospital has been a world leader in the use of fast neutrons for cancer treatment and if clinical trials at both centres are as successful in the future as they have been to date, the Department of Health could well be persuaded to make a big investment in this type of treatment.

The first cyclotron in the world to be entirely devoted to medical research was installed at Hammersmith in 1952. After extensive research in the laboratory, clinical trials in the treatment of advanced cancers were started in 1969. Randomised trials conducted alongside conventional programmes of X-ray treatment showed promising results in the treatment of cancers situated in the head and neck, although patients often died from secondary cancers which had spread before the primary source could be destroyed.

Radiotherapy in this area works on the principle that malignant tissue is more sensitive than healthy tissue to destruction by X-rays and γ rays. Where there is a low oxygen concentration in the tissue, caused perhaps by the presence of the tumour, there is, however, a reversal of the effect and the cancer is often able to grow again after partial destruction. The effectiveness of neutron therapy depends on the ability of the neutrons to destroy cancer cells even in the absence of oxygen.

After closely following the trials at Hammersmith, workers in the United States decided a year ago to adapt three of their large cyclotrons used in physical research for cancer treatment and other centres will be established in France, Germany, the Netherlands and Japan.

The Edinburgh cyclotron will produce neutrons with twice the energy available at Hammersmith and will be made more versatile by the inclusion of a steering device which will allow a site to be attacked from several different positions.

The new installation will be an outstation of the MRC's Hammersmith Cyclotron Unit, which is directed by Mr Derek Vonberg. The clinical programme will be directed by Professor W. Duncan of the Department of Radiotherapy (University of Edinburgh) and the clinical trials (which at Hammersmith were led by Dr Mary Catterall) will be supervised by a special committee on neutron therapy of the MRC, on which the Cancer Research Campaign will be represented.

What is wasted?

EVEN when we can actually lay hands on fuel for motor systems and power generation we waste a vast amount of the energy potential contained in the raw materials. In his Inaugural Lecture at the University of Leeds, Professor Alan Williams examined Britain's power station wastage and compared the fuel efficiencies of various methods of transport.

He finds that the average power station supplying 2,000 MW of electricity also produces 4,000 MW in steam and warm water which is generally not put to any use, so that even during the energy crisis Britain can only benefit from 45% of all fuel actually burnt.

The average engine is still less than 20% efficient in converting fuel into useful motive power and a comparison of different transport methods in terms of passenger miles per gallon showed a fully loaded train to be ten times more efficient than a jet aircraft and fifteen times more efficient than Concorde.

Professor Williams, Livesy Professor of Fuel and Combustion Science, considers that the efficiency of liquid fuel engines and power stations is capable of being improved by a further 10%. Of the 25,377,000 tons of liquid fuel used for transport in 1972, something like 74% was used by road vehicles operating at low efficiencies. Although in theory a diesel engine might have a thermal efficiency of 37% and a petrol engine 25% (17% in the case of a Wankel), in fact only 15% to 20% of their fuel is converted into useful energy.

Bringing NASA down to Earth

Colin Norman, Washington

BURIED in the pile of bills, resolutions and impeachment motions facing members of the US Congress who returned to Washington last week is a little-noticed item which could radically alter the National Aeronautics and Space Administration (NASA) and create yet another new focus for federal science policy-making. The bill, which was introduced into the Senate last September, is likely to be the subject of public hearings in February and the topic of considerable debate thereafter.

Called the "Technology Resources Survey and Applications Act" and numbered S2495, the bill would give NASA some \$200 million a year to help apply science and technology to pressing domestic problems, partly by enlisting the help of the depressed aerospace industry. It would also establish a Cabinet-level committee to survey the United States' scientific resources, and to plan how those resources could best be used to improve such items as health care delivery, sewage treatment and other domestic services.

The bill would thus greatly extend NASA's range of activities in fields other than space research, and it would add considerable impetus to the much-discussed move toward spending federal research and development dollars on more socially useful projects. But it is also likely to run into opposition from the Administration, and its prospects are uncertain.

In many respects, the bill is similar to Senator Edward M. Kennedy's National Science Policy and Priorities Act, which was passed by the Senate in 1972 but which died from neglect in the House of Representatives. Kennedy's bill would, however, have placed responsibility for supervising and sponsoring such domestic research and development programmes in the National Science Foundation—at best an unlikely location, but one which would have placed the whole effort under the jurisdiction of Kennedy's NSF Subcommittee. NASA is clearly a more logical home for such an enterprise because it already has an office concerned with technology utilisation, but even so, some bureaucratic turf fighting would be inevitable if the bill were passed in its present form.

For one thing, the National Science Foundation is now supposed to have responsibility for surveying the government's efforts in science and technology and for advising on how federal money should be spent on research and development. It would be unlikely to yield a good part of that responsibility

to the Cabinet-level committee proposed in the bill. And there is also the problem that the Administration would not take kindly to such a large spending commitment in such difficult financial times.

What are the chances that the bill will be passed? Staff members of the Senate Committee on Aeronautical and Space Sciences are hoping to get some hearings underway in February. But one problem is that the bill has also been referred to the Senate Commerce Committee whose chairman, Warren Magnuson, is the bill's chief sponsor. Unless some formula can be worked out for both committees to consider the legislation simultaneously, its passage through the mill will be slowed down. Moreover, even if the bill is passed by the Senate before Congress adjourns for the October Congressional elections, there is no sign of interest in the House of Representatives. The House Science and Astronautics Committee, for example, has its plate full with energy matters, and would be unlikely to take up the bill in time to get it passed by the House this year.

Nevertheless, the bill is likely to spark considerable discussion in the scientific community, just as Kennedy's bill did two years ago.

No astronomical ghosts laid

Edward Phillips

Dr David Evans, the Welsh-born optical astronomer, sees himself as the *enfant terrible* of British astronomy, although some would say *vieux terrible*.

After many years in British and South African astronomy he now works at the University of Texas, but last week he was in London and at the monthly meeting of the Royal Astronomical Society. And for once the society forsook its normal academic fare to allow Dr Evans to present his views on the management of British optical astronomy.

Dr Evans seemed to be attributing what he called "the depressed state of British optical astronomy" to two factors. First, he blamed "overbureaucratization" which he said resulted in the voice of optical astronomy emanating from administrators instead of from the scientists working at the observatories. And, second, he recognised "an unfortunate relationship between optical and theoretical astronomers", there being a tendency to disparage any work which originates too far from what he termed the Cambridge-Caltech axis.

All this was greatly enjoyed by the younger astronomers present, while their elders were notably less amused. But after this cracking start Dr Evans lapsed into a panegyric for astronomy at the University of Texas, in particular the McDonald observatory at Fort Davis which he put forward as the ideal site for the proposed Northern Hemisphere Observatory.

In the circumstances the president of the society, Professor D. E. Blackwell, was probably being benevolent in ruling that there was insufficient time for discussion, although Sir Bernard Lovell was allowed to say a few words in support of British optical astronomy.

Unfortunately, though, this meeting, which could have served as a much needed exorcism after all the troubles that have beset British optical astron-

omy, fell far short of achieving any useful purpose. The Royal Astronomical Society is, however, planning a meeting where officials of the Science Research Council will be able to explain the latest policies in astronomy. Maybe this will be the occasion for casting out a few devils.

Keeping a non-stop world

HAVING sacked the Earth to the point where (arguably) we are virtually bankrupt of fossil fuels, it is good to see a politician draw the line at actually stopping the planet from spinning in the pursuit of new energy sources.

In a House of Commons debate on tidal power British Member of Parliament Mr Tam Dalyell asked whether or not the government was prepared to talk about tidal energy at an international level since, he observed, there is a serious question of reduced Earth spin if several countries embarked on such schemes. Mr Dalyell also asked whether or not there would be adverse effects on the ocean bed and whether such fears need be harboured if there were a two-basin estuary scheme.

His point in raising these questions, apparently, was to demonstrate that people who proposed tidal energy schemes were aware of the attendant anxieties and, he submitted, it would be silly not to express a certain sensitivity towards them. Which of course is true, though, it must be added, it would not be ever so silly since the estimated time required for tidal harness schemes to stop the Earth's spin is something in the region of 10 million years.

correspondence

Energy

SIR—The United States is feeling the energy pinch and is beginning to realise that petroleum, along with other critical materials, cannot last forever. This is causing us to intensify our efforts toward alternate energy sources and to examine how we can reduce consumption and increase reclamation. It is also causing critics, both locally and abroad, to accuse America of the 'mindless' utilisation of energy (*Nature*, 246, 116; 1973). Statistics are quoted about our small population using a third of the world's energy and implying that this energy is mostly wasted or used for personal comfort and enjoyment.

I will concede that we have wasted resources, most recently in Vietnam, and that we drive too much, have too much throw-away packaging, and more air conditioning than we really need. But I argue that the majority of the energy has been used productively. America has exported energy to the four corners of the world in the form of wheat, rice, soy beans, cotton, lumber, machine tools, tractors, airplanes, locomotives, and medicine.

We have exported American know how, fed and clothed both allies and enemies after World Wars I and II, and rebuilt their factories. Their factories today are more modern than our own. For much of this we have received little or no payment—yet these activities have

caused depletion of our soil, natural resources, and treasury. I wish our critics in England, France, Russia, Germany, Japan, China, and the Middle East would remember some of these things when preparing their editorials.

In some instances we may have been guilty of exploitation and of not minding our own business. But, when it comes to energy, perhaps we have not used more than our fair share, considering the billions of BTU's used to make or grow the products we have given away.

Yours faithfully,

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news and views

New view of deep earthquakes

CONVENTIONAL wisdom has it that an earthquake is a sudden rupturing across a fault plane, the total duration of the event being roughly the maximum distance along which slippage occurs divided by the rupture velocity of several kilometres per second. It is also very widely believed that a 'mechanism' can be associated with every earthquake—a quadrantal distribution of rarefactions and compressions related to the orientation of the fault plane and the slip vector. A paper by Dziewonski and Gilbert in this issue of *Nature* (page 185) raises some fascinating questions about this simple and highly successful model of earthquakes. It seems that deep quakes can be preceded by over a minute of slow compression.

For the past ten years it has been possible to study radiation patterns of earthquakes by the use of long period (10 to 100 s) seismometer recordings of body waves. The signal begins sharply—it seems to have both a precise starting time and an unambiguous polarity. The observed distribution of these polarities over a notional sphere surrounding the quake is in almost all cases entirely concordant with a 'double-couple' model of the event. The few exceptions are probably explicable in terms of propagation anomalies. True, Knopoff and Randall looked into the possibility that body wave polarity distributions from deep focus earthquakes were somewhat removed from those of the simple model, but their evidence was not very strong. Almost all seismologists regard the model outlined above as an adequate description of the observed seismic source.

Nevertheless, there are some nagging problems. Plate tectonics explains shallow earthquakes as a manifestation of rubbing, slipping and cracking near plate boundaries and for such events the double-couple model of the source is entirely appropriate. But when plates descend beneath island arcs they presumably end up by being assimilated at a depth of about 700 km. During this descent into the mantle the lithospheric material has in all probability to undergo phase changes. The surrounding mantle is believed to contain large phase transitions at 400 km and 600 km depth at each of which physical properties suffer a change of several percent. Since there are earthquakes at all depths in the descending plate, the obvious question is whether deep events, although apparently double-couple in nature, contain any constituent, say, of compression that might be associated with an explosive phase change. Dziewonski and Gilbert's results indicate that this is a real possibility.

For several years the authors have been analysing the oscillations of the Earth which follow very large earthquakes. They and others have developed highly sophisticated methods for extracting signals at periods of up to 1 h from the hundred or so seismic stations around the world. As a result of this work the broad structure of the Earth's interior is known with some confidence. Until recently, however, the inversion process was simply from the observed eigenfrequencies to physical structure. There is more available, however—amplitude and phase of each eigenfrequency is accessible with careful processing. This permits inversion for

the generating function, or source mechanism as a function of time. The solution from body waves for the source mechanism is a high frequency one, but free oscillations cover a lower frequency range. Does the earthquake look similar in appearance at lower frequency?

The answer is that it does not. Dziewonski and Gilbert look at two deep earthquakes in South America. They find, not surprisingly, that to the extent that their solutions can be interpreted as double couples they are in agreement with body wave solutions for the mechanisms. There seems, however, to be a substantial constituent of non-deviatoric stress released and this stress is dominantly compressive. Furthermore, the inversion indicates clearly that compressional energy is in both cases released from the vicinity of the event for at least 80 s prior to what is more conventionally called the earthquake—the time of double-couple radiation of high frequency energy. The release of stress seems to be very smooth and thus there is no record of high frequency signal from it.

This is a most exciting result. If deep focus earthquakes can have a compressive precursor then this may be a transition to a more dense phase. The authors point out that it is entirely possible that it is the heat generated by this transition which causes local melting and triggers the quake along a lubricated slip plane. They further suggest that there may be occasions when the compression proceeds without triggering a slip.

Care must be taken in contrasting deep and shallow quakes, for both of which precursory signals have now been recorded. In both cases it seems that earthquakes can occur through lubrication of the fault plane. For shallow events the lubrication seems to come through a long term change in the distribution of fluids in the source region. For deep events, if the authors' speculation is correct, the lubrication would arise from exothermic phase changes. Doubtless more will be heard of deep earthquakes in the next year or two.

D. D.

T cell Ig receptors—to be or not to be?

THERE are few subjects of investigation in cellular immunology which have attracted more controversy than the nature of the receptor for antigen on thymus-derived (T) lymphocytes. A good illustration of this is to be found in several recent articles, two of which by Roelants and colleagues, have appeared in *Nature* (247, 104 and 106; 1974).

The central question is whether T cells like B cells have surface bound immunoglobulin (Ig) receptors. Several groups of investigators have subjected lymphocytes to enzymatic radioiodination of their cell surface proteins, followed by disruption of the cell and precipitation by specific anti-immunoglobulin antisera. Using this method, Marchalonis *et al.* (*Transplant. Rev.*, 14, 1; 1973) claim to have detected and isolated significant quantities of surface Ig on T cells

and on thymocytes. Their conclusion is that T and B cells have similar amounts of surface Ig. Vitetta *et al.* (*Transplant. Rev.*, **14**, 50; 1973), however, using the same method, could only detect minute quantities of membrane associated Ig on thymocytes or peripheral T cells. Furthermore, if such cell suspensions were first treated to eliminate small numbers of contaminating B cells, then the 'pure' T cell suspensions did not subsequently yield any Ig.

Using a similar method, Grey *et al.* (*J. exp. Med.*, **136**, 1323; 1972) could not detect appreciable amounts of T cell Ig. Similarly, Lisowska-Bernstein *et al.* (*Proc. natn. Acad. Sci., U.S.A.*, **70**, 2879; 1973) were unable to detect T cell Ig on thymocyte or cortisone-resistant thymocyte cell suspensions which had been pretreated so as to kill any contaminating B cells or IgM-containing plasma cells. Antigen-activated T cells were found by labelling experiments with radioactive leucine and methionine apparently to synthesise μ and light Ig chains, but again, this was probably due to contamination by IgM-containing plasma cells. The conclusion is that most if not all T cell Ig is adsorbed material, a conclusion made more plausible by the recent demonstration that activated T cells have Fc receptors which can recognise certain Ig molecules by means of their Fc portion and bind them readily when they are complexed to specific antigen (Yoshida and Andersson, *Scand. J. Immun.*, **1**, 401; 1972). Thus, of four laboratories, using very similar or identical techniques, three maintain that little or no Ig is made by T cells, and only a little more is sometimes displayed on the cell surface.

By contrast, Roelants, Rydén, Hägg and Looor (*Nature*, **247**, 106; 1974) conclude not only that T cells have antigen-specific Ig receptors, but that they are also actively synthesised by the cells carrying them. The system used by Roelants *et al.* to demonstrate T cell antigen-specific Ig receptors is indirect, involving inhibition of antigen binding between T cells and highly radioactive antigen (which is visualised and quantitated by radioautography) after pretreatment of the cells with a polyvalent rabbit anti-mouse Ig serum.

The anatomic origin of the antigen binding cells was determined by Roelants *et al.* by immunofluorescent techniques involving anti- θ serum as a T cell marker, or anti-Ig serum, as a B cell marker (T cells evidently have too few surface Ig molecules to be readily visualised by this second technique). Using this combined immunofluorescent-radioautographic technique, Roelants *et al.* demonstrated a small number of

antigen-binding T cells to a synthetic antigen, which in turn could be inhibited by an anti-immunoglobulin serum, thus confirming previous observations (Roelants *et al.*, *J. exp. Med.*, **137**, 1060; 1973). But equally important, the authors go on to conclude that these surface Ig molecules are endogenously produced by the T cells themselves, and are not passively absorbed. The authors reasoned that if Ig molecules are passively absorbed, then after inducing their capping and internalisation by anti-immunoglobulin serum, the T cells should remain negative for cell surface Ig when incubated *in vitro* at 37°C. On the other hand, if endogenous synthesis of the Ig moieties is the rule, it should be possible to demonstrate the reappearance—and therefore 'recapping'—of the receptors. This second situation proved to be the case, and authors conclude their experiments demonstrate active synthesis of Ig receptors for antigen by T lymphocytes.

It should be pointed out that the validity of the interpretation is dependent on independent movement of cell surface components under the influence of capping stimuli. For example, it could be argued that T cell receptors which are intimately associated with passively-acquired surface Ig molecules could be 'co-capped' with the Ig molecules by the anti-Ig treatment. Roelants *et al.* say there is no such precedent for co-capping, but recently Schlesinger has cited unpublished observations from his laboratory indicating that H-2 antigens and C3 receptors on the cell surface of peritoneal macrophages can be co-capped (*Biomedicine*, **18**, 473; 1973).

It is difficult to reconcile the conflicting opinions which presently exist with regard to Ig receptor molecules on T cells. Fairly broad agreement is evolving that T cells do have surface Ig, but much less than B cells have; but whether these few molecules are passively acquired or are endogenously produced, now seems to be the centre of the controversy. Some of the experimental differences may eventually turn out to be related to the use of heterologous anti-immunoglobulin sera which all these experiments entail; although presumed to be 'monospecific', it is doubtful, even after rigorous absorption, whether such sera exist. A second factor which will have to be reckoned with is the part Fc receptors on certain types of T cells may be playing in the various experimental protocols. Basten *et al.* (*J. exp. Med.*, **135**, 610; 1972) have already cautioned others on the difficulties such receptors could cause in interpreting results of studies on antigen binding properties of B cells: this warning must now be extended to similar T-cell studies.

R. K.

A. D.

Growing hepatitis B virus

from our Medical Virology Correspondent

THE frustrating problem of propagating the elusive hepatitis viruses in tissue culture has been a major obstacle to defining the biophysical, biochemical and biological properties of these infectious agents and to the production of suitable vaccines. Hepatitis B antigen (Australia antigen), now generally believed to represent the outer protein coat of the virion, provides a convenient specific marker of infection with type B hepatitis virus. The availability of such a marker permitted some progress in tissue culture studies. Brighton and colleagues (*Nature new Biol.*, **232**, 57; 1971) for example, reported the induction of progressive non-cytocidal involvement of normal cellular components of monolayer cultures of human embryo liver cells after inoculation with a well-documented infective hepatitis B serum. Hepatitis B antigen was localised by immunofluorescence in the cytoplasm and in the nuclei of the cells. Smith and Francis (*Cancer Res.*, **32**, 1713; 1972) obtained similar results.

In subsequent studies, following the inoculation of supernatant fluid which had been passaged twice in culture, the outer coat (hepatitis B) antigen was demonstrated by immunofluorescence, using specific animal antiserum, in the cytoplasm of cultured liver cells. Only nuclear fluorescence was observed when human antibody was used (Zuckerman and Earl, *Vox Sang. Suppl.*, **24**, 123; 1973). Similar fluorescent changes were also noted by Coyne and her associates (*Proc. Soc. exp. Biol. Med.*, **138**, 1051; 1971 and London *et al.*, *Can. med. Ass. J.*, **106**, 480; 1972) in liver cells cultured from biopsies obtained from two patients with circulating hepatitis B antigen. In addition the antigen was detected by radioimmunoprecipitation in the supernatant fluid of a few cultures which had been serially passaged.

The morphological and functional capacity of cells maintained in organ culture offers a system for cultivating viruses which simulates conditions in the intact host. Such techniques have proved valuable for cultivating viruses which are difficult to grow in conventional cell monolayers. Cultured fragments of human inguinal lymph nodes were incubated by Jensen and colleagues (*Exp. molec. Path.*, **13**, 217; 1970) with serum containing hepatitis B antigen from a drug addict admitted to hospital with hepatitis. On the basis of serological tests and electron microscopy of the supernatant fluid and examination of ultrathin sections of the explants, it was concluded that some lymph node organ

cultures may support the *in vitro* production of hepatitis B antigen. More recently, Zuckerman and associates (*Nature*, **236**, 78; 1972) reported that hepatitis B antigen may be produced in human embryonic liver organ cultures. A progressive increase in the titre of hepatitis B antigen, measured by several techniques and confirmed by immune electron microscopy, was shown with a limited number of sera, but only one successful passage of harvested material was accomplished.

Shikata (*Jap. J. exp. Med.*, **43**, 231; 1973) demonstrated hepatitis B antigen by immunofluorescence for 4 weeks in organ cultures of human foetal liver tissue grown in a Rose chamber. Specific fluorescence was also found in the cytoplasm of spindle-shaped cells growing out from the original liver tissue and it was considered that these fibroblast-like cells phagocytosed the antigen produced by the hepatocytes. The culture medium was not examined and serial passage was not attempted. Taylor and Kelen (*Proc. Can. Fed. Biol. Soc.*, **15**, Abst. 499; 1972) used liver organ and monolayer cultures from recently killed non-human primates. Fluid harvested 8 days after primary inoculation of Rhesus monkey liver organ culture with serum containing hepatitis B antigen was found to contain readily detectable antigen as well as a substantial increase in the proportion of the 42 nm (Dane) particles. Passage of harvested fluid was not achieved.

More exotic studies were lately described by Banatvala and Payne (*Lancet*, **i**, 1359; 1973), who found that sera from patients with hepatitis B infection and sera from some carriers of the antigen induced necrotic changes in the leaves of tobacco plants. Neutralisation tests and preheating the inoculated sera in a boiling water-bath inhibited the necrotic effects in the plants. But again transmission of the virus was not demonstrated.

Although many of these reports are encouraging and suggest that hepatitis B virus may be cultured with difficulty in mammalian cells, consistent serial passage of this virus had not been attained. Panouse-Perrin and her colleagues now report (*Biomed. Express*, **19**, 442; 1973) preliminary results of the cultivation of hepatitis B virus in a human diploid cell line derived from liver biopsies obtained from antigen-negative children undergoing surgery. Electron microscopy revealed the presence of 20 nm icosahedral particles with ninety-two capsomeres in the cell and in the supernatant fluid of the cultures after serial passage. After the fourth passage numerous clusters of capsids, empty icosahedrons about 20 nm in diameter, and electron dense particles measuring 25 to 27 nm were present. These particles are

considered to be similar, if not identical, to the internal component or inner core of the 42 nm (Dane) particle (Almeida *et al.*, *Lancet*, **ii**, 1225; 1971). After fourteen passages cellular degeneration occurred and particles similar to the three morphological entities generally associated with hepatitis B antigen were visualised.

Serological tests, including radioimmunoassay, on supernatant fluid obtained after ultrasonic disintegration of the cultures proved negative for hepatitis B antigen; but precipitin lines were obtained by counter-immunoelectrophoresis when the fluids were tested against sera from patients in the early phase of convalescence from hepatitis B infection. The precipitin lines may have resulted from the interaction between the inner core antigen produced in the cultures and the antibody in serum of patients recovering from hepatitis (Almeida *et al.*, *loc cit*; and Hofnagle *et al.*, *Lancet*, **ii**, 869; 1973). If these observations can be confirmed and extended then a giant leap forward in hepatitis research has just taken place.

Genetic variation of histones

from our Molecular Genetics Correspondent

ONE of the most interesting observations to have been made in cell biology during the past couple of years is the conservation of amino acid sequences which seems to prevail among the histone proteins. Although only a small number of histones has been sequenced at present, one of the five fractions has an almost identical sequence in several species related only distantly in evolution; its extent of sequence conservation, in fact, seems to be the highest yet observed in any protein. Electrophoretic analysis of histone fractions suggests that conservation of the other four fractions may also be appreciable, although not as extensive as that of histone IV. The apparent homogeneity of sequence in each histone fraction has important consequences for the structure of the chromosomes and, a less fortunate consequence, means that investigations relying upon genetic variation may be rather difficult. In the November issue of the *Proceedings of the National Academy of Sciences*, however, Stout and Phillips (**70**, 3043; 1973) report the existence of genetic variations in the lysine rich histone fraction I of maize (*Zea mays*).

The lysine rich group of histones is the only one of the five histone fractions that shows any internal variation; it seems to consist of several proteins whose sequences are identical in most of their length but vary at what are

probably the amino acid residues present at only a small number of positions. Stout and Phillips have now shown that there may be a genetic polymorphism in these subfractions, with different groups of alleles coding for the lysine rich histones in different inbred lines of maize.

Slight differences in the electrophoretic mobilities of the subfractions of the lysine rich histones were first noticed when a line of maize carrying the *sticky* mutation—which produces sticky chromosomes at meiotic anaphase in pollen cells—was compared with another line lacking the mutation. The rationale for these experiments was that the *sticky* mutation might have as its molecular effect the alteration of properties of one of the chromosomal proteins. In genetic mapping experiments, the variation in one lysine rich histone subfraction (Ia) behaved as though coded by a single Mendelian-segregating gene, independent of the *sticky* locus. Another lysine rich subfraction showing genetic variation (Ic) seems to segregate independently of the locus controlling Ia variation.

In spite of the initial rationale for these experiments, the meiotic mutant *sticky*, and also another meiotic mutant *elongate*, do not seem to control a histone protein. But a comparison of several inbred maize lines showed that each exhibits a specific variation in its histone I subfraction pattern, consistent with earlier experiments which have shown that this fraction is the least conserved in evolution. The only other histone fraction which has been shown to comprise genetic variation is the unique fraction V, which replaces the lysine rich fraction I in avian erythrocytes. Greenaway and Murray (*Nature new Biol.*, **229**, 233; 1971) found that it has two subfractions under genetic control, one with a glutamine at position 15 and one with an arginine. These experiments however, did not show whether the segregating locus comprises only a single gene, as is the case in maize.

The demonstration that variation in lysine rich subfractions in maize may be due to single unlinked mendelian factors contrasts with conclusions derived from hybridisation with DNA of the messenger RNA fraction coding for the histones; these biochemical experiments have suggested that many genes—which in *Drosophila* may be clustered together—might code for each histone protein. But, of course, until the different histone I variations of maize have been sequenced, it is not possible to be certain that the gene controlling their segregation is the structural locus; although the lysine rich histones in general do not seem to suffer modification, until proved otherwise it remains possible that the segregating locus controls modification rather than synthesis *per se*.

The homogeneity of each histone fraction is most consistent with models which suppose that each protein is controlled by a single genetic locus. Although, of course, it is possible to construct models for the organisation of the genome which reconcile unifactorial genetic inheritance with gene multiplication, experiments with messenger RNAs purified for other gene products suggest that the presence in the genome of a single structural gene for each protein may be the more general phenomenon. The histone system at present poses more problems than it resolves, for the striking conservation of histone sequences in disparate organisms remains inexplicable; clearly, the whole sequence of (at least) histone IV is critical for the organism, variation of even a single amino acid being prohibited, which implies that there may be important similarities in chromosome structure in all eukaryotes. But although it is obvious that conservation of histone structures conveys an important message about the organisation of the genetic apparatus, the nature of this message remains elusive.

Diapause regulation by juvenile hormone

from our *Insect Physiology Correspondent*

WHEN the hormonal control of moulting and metamorphosis in insects was defined about forty years ago it was suggested that the periodic arrest of growth, or reproduction, that occurs in many insect species and which is called 'diapause', was the result of an arrest of hormone secretion—since a state closely resembling natural diapause could be induced by the experimental exclusion of the growth hormones. Experiments on a wide range of insects have largely substantiated this idea. Sometimes it is the neurosecretory cells in the brain which are inactive; sometimes the prothoracic glands. In adult insects the failure of egg development during diapause results sometimes from a failure in the secretion of the juvenile hormone by the corpora allata, as in the potato beetle *Leptinotarsa*; sometimes from an arrest in the neurosecretory cells of the brain, as in overwintering mosquitoes.

But in this story the juvenile hormone has come to occupy a somewhat equivocal role. It was noted by the Japanese workers Fukaya and Mitsuhashi that in the caterpillar of the rice stem borer *Chilo*, exclusion of the corpus allatum induced the diapausing insects to resume their development. These authors therefore inferred that the juvenile hormone was acting as a 'diapause factor'.

Yin and Chippendale (*J. Insect Physiol.*, **19**, 2403; 1973) have now re-

investigated this problem in another caterpillar, the southwestern corn borer *Diatraea*. It has been known for some years that in certain Lepidoptera the juvenile hormone experimentally applied may serve as a stimulus to the prothoracic gland to induce secretion of ecdysone and thus to bring about moulting in caterpillars deprived of the brain. This happens in *Diatraea*; and when sufficient juvenile hormone is present the insect undergoes a 'stationary moult', that is, it retains its larval form. Whereas, if only a very small amount of juvenile hormone is present the caterpillar changes to a pupa when it moults.

It has long been known that in Lepidoptera and other insects with a complete metamorphosis a high titre of juvenile hormone causes retention of larval characters, a lower titre signals the evocation of pupal characters, and in the absence of juvenile hormone adult characters appear. Yin and Chippendale produce good experimental evidence to support their novel hypothesis that the diapause state in this particular insect, *Diatraea*, is evoked by yet another defined level of the juvenile hormone titre, slightly lower than that required for a larval-larval ecdysis. In some of the diapause larvae the juvenile hormone titre is apparently high enough to activate the prothoracic gland and thus to promote stationary ecdyses from time to time.

Liquid crystals at Bangalore

from a *Correspondent*

To commemorate the twenty-fifth anniversary of the founding of the Raman Institute at Bangalore in India and to highlight the present role of the institute as a centre for liquid crystal research, Professor S. Chandrasekhar organised an international conference on liquid crystals from December 3 to 8. The conference was preceded by a week's winter school on liquid crystals led by four guest speakers—P. G. de Gennes (College de France, Paris), G. W. Gray (University of Hull) and G. H. Brown and A. Saupe (Liquid Crystal Institute, Kent State University, Ohio).

P. G. de Gennes opened the meeting by reviewing developments in the mixed elastic behaviour of smectics A phases. He dealt with continuous deformations, singular lines and dynamical aspects of smectic A to nematic transitions. This contribution and that by G. Durand (Orsay Liquid Crystal Group) on Rayleigh scattering in smectic and nematic materials reflected the high degree of interest in smectic phases and their polymorphism. A. de Vries (Kent State University) in his paper on X-ray

studies of thermotropic liquid crystals then expounded his classification of smectic polymorphs based on X-ray diffraction patterns. This system of classification obviously does not satisfy everybody, and it is clear that there is not yet adequate structural information about the eight smectic polymorphs, for the existence of which experimental evidence has been proposed, to form the basis of a thoroughly satisfactory classification scheme. As yet uncategorised smectics probably exist, for example, the smectic phase of 4'-n-octyl-4-cyanobiphenyl described by Gray.

Areas of research which may contribute to solving the problems of smectic polymorphism are those concerned with elucidating molecular motion and diffusion in mesophases. Such investigations featured in presentations by B. J. Bulkin (Hunter College of the City University of New York) on infrared optical activity as a probe of nematic order, by J. M. Schnur (Naval Research Laboratory, Washington) on Raman spectroscopy of nematic and smectic mesophases, by R. Blinc (University of Ljubljana) on soft modes in nematic and smectic liquid crystals and by J. A. Janik (Institute of Nuclear Physics, Cracow) on estimations of rotational correlation times for nematic phases.

Several contributions were made by Chandrasekhar's liquid crystal group at Bangalore, and the following titles reflect the range of physical studies being made at this centre: determination of twist elastic constants of nematic liquid crystals; pressure induced mesomorphism; theory of reflection and transmission by cholesteric films; anomalous transmission in absorbing cholesteric liquid crystals; circular dichroism in absorbing mixtures of right and left handed cholesterics; role of permanent dipoles in nematic order; statistical thermodynamics of the nematic liquid crystal surface; new types of electrohydrodynamic flow patterns in nematic liquid crystals and nuclear magnetic resonance spectra of molecules oriented in thermotropic and lyotropic nematics. Several of these contributions represented joint investigations with the National Aeronautical Laboratory, Bangalore or the Bhabha Atomic Energy Centre, Bombay.

Other presentations of importance were given by Saupe on electrohydrodynamic instabilities—a valuable review illustrated by striking photomicrographs—by D. Demus (Martin Luther Universität, Halle) on calorimetric investigations of liquid crystals, by F. M. Leslie (University of Strathclyde) on a theoretical analysis of distorted twisted orientation patterns in nematics and by E. J. Ambrose (Chesster Beatty Research Institute, London)

on the liquid crystalline properties of biological microfilaments.

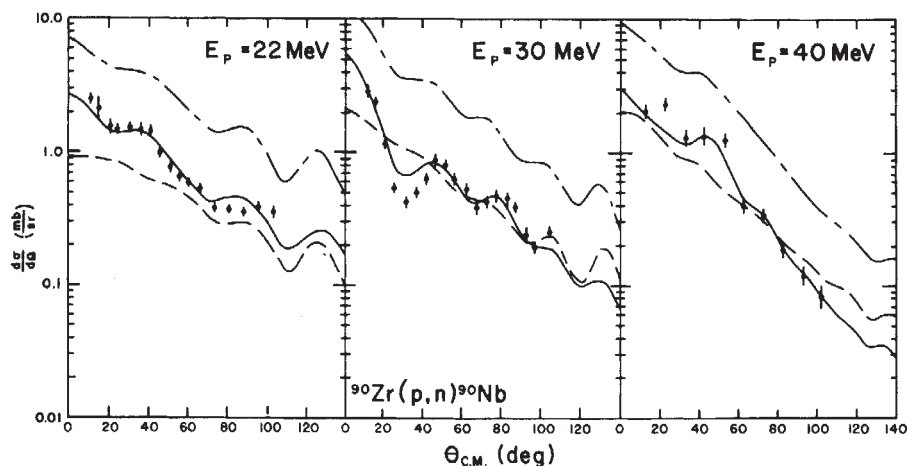
Present interest in liquid crystals for electro-optical displays was reflected in the contributions by J. Billard (Collège de France and Université de Lille), who illustrated how eutectic compositions can be calculated for mixtures of mesogens, and by Gray who described further developments relating to the colourless, stable, room temperature smectics, nematics and cholesterics provided by 4'-n-alkyl- and 4'-n-alkoxy-4-cyanobiphenyls. Such compounds and their mixture (including those incorporating *p*-terphenyl analogues) provide excellent materials for twisted nematics and cholesteric-nematic phase change displays. S. Kobayashi (Saitama Institute of Physical and Chemical Research, Japan) also discussed off-90° twisted nematic films for use as voltage dependent colour controlled devices and orthogonal twisted nematic films, with coloured polarisers, for producing a range of coloured displays.

Two-step nuclear reactions

from our Nuclear Theory Correspondent

THERE has recently developed a growing awareness of the importance of multi-step processes in nuclear reactions. These are of two types: reactions involving pre-excitation of the target nucleus or post-excitation of the residual nucleus by inelastic scattering and reactions involving sequential transfer. If the direct process is inhibited in any way, the multistep processes can account for a significant fraction of the measured cross section, so that any conclusions concerning the structure of the participating nuclei based on the assumption that only the direct one-step process contributes may be gravely in error. Among the many possible multistep processes the two-step ones are the easiest to calculate, and detailed theories have now been programmed for electronic computation.

A relatively simple example of the importance of including two-step processes is provided by the (p,n) reaction to the isobaric analogue state of the target nucleus. This final state has essentially the same structure as the ground state of the target apart from a reversed isospin vector for one of the target nucleons: a neutron is replaced by a proton in the same shell-model state. On a simple view the reaction thus proceeds entirely by the isospin term in the nucleon-nucleus optical potential. The total wave function is thus expressible as a sum of the wave-function in the elastic channel and that in the (p,n) or quasi-elastic channel. Use of the Schrödinger equation with an



Differential cross sections for the (p,n) reactions to the isobaric analogue states compared with calculations with the one-step process only (—), the two-step process only (---) and the one-step and two-step processes combined (— · —).

isospin-dependent potential that can cause transitions from the elastic to the quasi-elastic channel gives a pair of coupled differential equations (called the Lane equations) for the two wave functions. These can be solved to give the observable cross sections in terms of the parameters of the potentials used. It is then possible by comparing calculations with the experimental data to obtain the best values of the parameters of the potential, in particular of the isospin term. Such analyses have been moderately successful in that with suitable choice of parameters it is possible to fit the data fairly well, and to obtain an estimate of the isospin potential.

These analyses assume that the reaction is a direct one-step process from the incident to the final state. It is, however, possible that the reaction can take place in two or more stages, and this can be investigated using a microscopic model of the interaction in which the contributions of all nucleons outside closed shells are explicitly included.

This model has recently been used by Rickertsen and Kunz (*Phys. Lett.*, **47B**, 11; 1973) to study the contribution to the quasi-elastic cross section due to successive particle transfer: instead of direct (p,n), there is a (p,d) pickup followed by (d,n) stripping, leading to the same final state as before. The formalism for this process is much more complicated than for the one-step process, but it has been worked out to give a set of coupled differential equations that can be solved on a computer.

In these microscopic interactions the essential parameters are those of the effective nucleon-nucleon interaction, expressed as a sum of central and isospin terms, together with those of the potentials describing the distortion of the incident and outgoing waves and the shell model used for the initial and final nuclei.

Rickertsen and Kunz have made a series of analyses of (p,n) quasi-elastic

cross sections using both the one-step process on its own and then the one-step with the addition of the two-step. It was found, as shown in the figure, that the one-step alone is not always very successful, even if the absolute magnitude is adjusted by altering the strength of the isospin term but that with the addition of the two-step process the agreement is very satisfactory. It is notable that the one-step contribution on its own gives a cross section greater than that observed, so there is considerable destructive interference when the two-step process is included as well.

It is also found that the strength of the isospin term in the effective nucleon-nucleon interaction varies over a wide range from about 15 MeV to 33 MeV if the data are analysed with the one-step process alone, whereas if the two-step process is included the values of the strength cluster much more closely around a mean value of about 29 MeV. The contributions of the two-step process differ from nucleus to nucleus depending on their individual shell structure and they are such as to account for the data, when combined with the one-step process, with parameter values having a more uniform behaviour from nucleus to nucleus.

Thus both the angular distributions and the absolute magnitudes of the (p,n) quasi-elastic cross sections are much better accounted for by the inclusion of the two-step process. It is physically very plausible and gives a more coherent account of the experimental data. The contribution of a two-step process involving pre- or post-excitation of the target or residual nucleus by inelastic scattering was also calculated and found to be quite small.

The possibility of such two-step contributions to nuclear reactions must therefore be carefully considered, and a full calculation made if one is to obtain a detailed and accurate account of the data.

Upper mantle model variations

from our *Geomagnetism Correspondent*

As the newly-formed oceanic lithosphere spreads away from its ridge source it gradually cools and contracts; and the elevated, rugged topography characteristic of the ridge ultimately gives way to the lower, smoother surface beneath the sediments of the ocean basin. If the cooling, contracting lithosphere were to move over a laterally uniform asthenosphere, the only other factor governing the ridge shape and dimensions would be the spreading rate. Thus several workers, beginning with McKenzie (*J. geophys. Res.*, **72**, 6261; 1967), have constructed thermal models involving the structure of the lithosphere across ocean ridges in which particular attention has been paid to the effect of spreading rate on the topographic profile. In all such models the spreading rate was found to affect principally the horizontal scale of the topography, with an increase in spreading rate producing a lengthened section but almost no variation in vertical dimensions. But as Haigh (*Geophys. J.*, **33**, 405; 1973) has now pointed out with reference to the North Atlantic, although the differences in horizontal scale predicted by previous thermal models exist, there are also variations of ridge and basin structure with latitude which cannot be attributed to differences in spreading rate. He thus concludes that the basic premise of a uniform asthenosphere is wrong, and that instead the temperature at a given depth in the asthenosphere must vary laterally.

The latitudinal variations which require explanation have been known in general terms for some time, the particular data now analysed by Haigh being derived from Atlantic bathymetric and gravimetric profiles obtained as long ago as 1964–1965. From these it is clear, for example, that between low latitudes and Iceland the depth at which the topographic profiles across the Atlantic ridge system become level in the ocean basins decreases northwards from 5.5 km at 30°N to 3.0 km and 60°N. Because the ocean basin level is not reached at the same distance from the ridge throughout the whole latitude range, there may be a suspicion that the variation of basin depth with latitude arises from differences in lithospheric age. No such explanation is possible here, however, because all the ages involved are greater than 60 million years; and as Sclater *et al.* (*J. geophys. Res.*, **76**, 7888; 1971) have shown, changes in elevation beyond this age are negligible. Nor can the marked northward decrease in basin to ridge crest elevation between the Azores and Iceland (the region studied in detail

by Haigh) be explained away quite so simply. On the other hand, spreading effects are significant when it comes to ridge cross-sectional area. Even so, when the effects of variable spreading rate on horizontal scale are adjusted throughout to the spreading rate at 43°N, there is still found to be a marked decrease in ridge cross-section area northwards from the Azores.

To investigate the causes of these variations Haigh has constructed a model in which, in common with previous thermal models, the lithosphere is regarded as a slab of uniform thickness resting isostatically on an asthenosphere assumed to be at constant temperature across the ridge. As new material at this temperature is added gradually to one end of the slab, the slab increases in length so that a point on the lithosphere moves away from the hot edge at a rate governed by the rate at which new material is added. Solution of the relevant differential equations in conjunction with assumed values of the physical parameters involved then gives the temperature distribution in the lithospheric slab for any given slab thickness and composition. But in order to obtain an elevation profile from the temperature distribution it is necessary to know the density of the lithospheric material at the appropriate temperatures and pressures—and this involves all the usual problems in dealing with a body whose composition is unobtainable by direct sampling.

Haigh's approach has been to assume that the upper mantle comprises basalt and dunite, with or without water, and either in the ratio 1:3 as suggested by Ringwood (*J. geophys. Res.*, **67**, 857; 1962) in his 'pyrolite' model or in the ratio 1:1 as proposed by Press (*Science*, N.Y., **165**, 174; 1969). The end result is then a series of calculated ocean ridge profiles (elevation-distance graphs out to 900 km from the ridge) for the two different basalt-dunite compositions, with or without the involvement of water, and for various lithospheric thicknesses. In addition, to account for possible heat transfer by radiation, a further variable in the form of a conductivity increase above 800°C has been introduced.

The general conclusion derived from a comparison of the calculated and observed profiles was that many models give too great a ridge elevation unless the allowed ranges of lithospheric thickness and composition are limited. More specifically, a composition involving a basalt-dunite ratio of 1:3 was found to be more likely than one with a ratio of 1:1; and the mantle must contain a small amount of water in order to allow the decoupling between the lithosphere and asthenosphere to occur at a lower temperature than is necessary for a dry model. In addition, models involving a

conductivity increase above 800°C were in poor agreement with observation, having too much elevation at the ridge axis and not enough on the flanks. And, finally, raised temperatures in the asthenosphere were found to give an upward migration of phase boundaries, resulting in a thinner lithosphere and thus a reduction of ridge cross section and elevation.

In the context of the observed variations with latitude between the Azores and Iceland the last of these conclusions is particularly significant, for it implies an explanation. Haigh's next step was thus to compare the North Atlantic topographic profiles with profiles calculated for a range of lithospheric thicknesses. From a least squares fit between each observed profile and the calculated profiles with different thicknesses, it became clear that the lithosphere thins from 85 km at 43°N to 64 km at 61°N (although these thicknesses increase by about 10 km on average if the presence of sediments is taken into account). In view of the conclusion that a thinned lithosphere is associated with raised temperatures in the asthenosphere, Haigh thus suggests that the temperature at a given depth in the asthenosphere gradually increases northwards from the Azores to Iceland. He is also able to show that such a lateral temperature variation in both the asthenosphere and lithosphere can explain the northward rise of the whole basin and ridge system with respect to sea level, and not just the variation of those parameters such as ridge cross section and elevation which are measured with respect to the ocean basin level.

In thinking about a northward temperature increase towards Iceland it is tempting to see Iceland as the region of maximum temperature and thus as either the site of an uprising mantle plume as suggested by Morgan (*Nature*, **230**, 42; 1971) or of a convective overturn as suggested by Bott (in *Implications of Continental Drift to the Earth Sciences*, Academic Press, 1973). If Iceland really is the temperature focus, the northward variations between the Azores and Iceland should be mirrored by similar variations southward between the Arctic and Iceland. At present there are few data from north of Iceland, and so little can be said about this except that Haigh claims some support for the dimensional increase in the ridge to the north in the work of Johnson and Heezen (*Deep-Sea Res.*, **14**, 755; 1967). For the time being, Haigh is obviously unwilling to be drawn into a detailed argument over mantle plumes, and rests content with the comment that his model is consistent with uniquely higher temperatures beneath Iceland. But he emphasises that his general conclusions, if valid, should be applicable to other ridge systems.

Flux of Gases across the Air-Sea Interface

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This article describes the use of a two-layer model to estimate the flux of various gases across the air-sea interface.

In the study of gas exchange between air and water, the interface between the two phases is often considered as a two-layer (film) system^{1,2}. The main body of each fluid is assumed to be well mixed; the main resistance to gas transport coming from the gas and liquid phase interfacial layers, across which the exchanging gases transfer by molecular processes (Fig. 1).

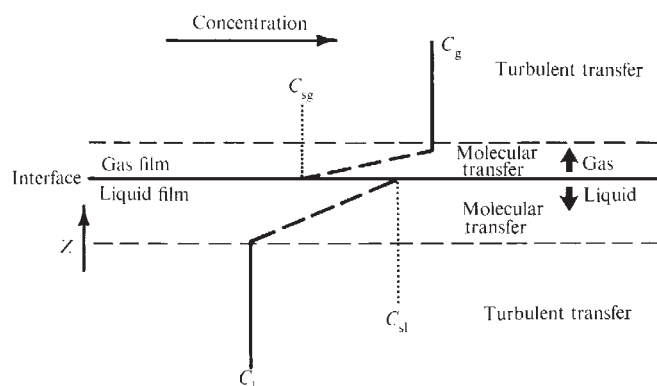


Fig. 1 Two-layer model of a gas-liquid interface.

That this simple model adequately describes processes at the interface must be open to question. The idea of such discontinuities close to the interface is physically unrealistic and, even under given conditions of turbulence in both phases, the layer thicknesses will vary both spatially and temporally. In spite of these difficulties, the film model is useful in visualising processes at the interface and for simplifying theoretical calculations of gas exchange rates. Furthermore, predictions based on the film approach often show little or no difference from those derived using more sophisticated models².

Since transport through the layer system is by molecular diffusion, Fick's first law in the one-dimensional form (with z as the vertical direction) is applicable.

$$F = -D \frac{\partial c}{\partial z} \quad (1)$$

where F is the flux of gas through layer; D the coefficient of molecular diffusion of gas in layer material; and c the gas concentration.

The more usual form of equation (1) for gas exchange studies is

$$F = k \Delta c \quad (2)$$

where, Δc is the concentration difference across the layer (thickness z) and

$$k = D/z \quad (3)$$

k is called the exchange constant and has dimensions of velocity; it is a measure of the flux of gas per unit concentration gradient. The value of k depends on many factors, of which the degree of turbulence in the fluids on both sides of the interface and the chemical reactivity of the gas are among the most important. The reciprocal of the exchange constant is a measure of the 'resistance' to gas transfer, and has dimensions of $[T] [L]^{-1}$. The total resistance to the exchange of any gas will be due to a combination of the resistances of the gas and liquid phases. Any surface contaminants will contribute to the resistance of the phase in which they occur.

Applying equation (2) to the two-layer situation shown in Fig. 1, and assuming that transport of gas across the interface is a steady state process, it follows that

$$F = k_g(c_g - c_{sg}) = k_l(C_{sl} - C_l) \quad (4)$$

where k_g and k_l are the exchange constants for the gas and liquid phases, respectively.

If the exchanging gas obeys Henry's law, then

$$c_{sg} = H C_{sl} \quad (5)$$

where H (the Henry's law constant) = [equilibrium concentration in gas phase (g per cm³ of air)]/[equilibrium concentration of unionised dissolved gas in liquid phase (g per cm³ of water)].

Eliminating c_{sg} and C_{sl} between equations (4) and (5),

$$F = (c_g - H C_l) / (1/k_g + H/k_l) = (c_g/H - C_l) / (1/k_l + 1/Hk_g) \quad (6)$$

which can be written as

$$F = K_g(c_g - H C_l) = K_l(c_g/H - C_l) \quad (7)$$

where

$$1/K_g = 1/k_g + H/k_l \quad (8)$$

and

$$1/K_l = 1/k_l + 1/Hk_g \quad (9)$$

Hence, the total resistance (R) expressed on either a gas phase ($1/K_g$) or a liquid phase ($1/K_l$) basis, depends on the exchange constants of the individual phases and the value of the Henry's law constant for the gas concerned.

For convenience in using equation (9), $1/k_l$ may be written as r_l , and $1/Hk_g$ as r_g ,

$$R_l = r_l + r_g \quad (10)$$

The numerical values of r_l and r_g for any gas indicate the relative importance of gas and liquid phase resistance for

exchange of that particular gas. Except in special cases, it is difficult to measure individual resistances, and most field and laboratory studies have measured only the total resistance. As will be shown, however, for many gases the resistance of one phase tends to predominate. In such cases the total measured resistance is, for all practical purposes, numerically equal to the individual resistance of the rate controlling phase.

Factors Affecting Resistance

Gas phase resistance (r_g). In common with evaporation-condensation of any appreciably pure liquid, the liquid phase resistance should be negligible for water molecules crossing the air-sea interface³. This means that for water $r_l=0$, and exchange of H_2O across the interface will be controlled by processes in the gas phase. In this case the total measured resistance (R) will be numerically the same as the resistance of the gas phase (r_g). Laboratory studies indicate that the value of $k_{g(H_2O)}$ increases linearly with wind velocity⁴. Using the field data of Schooley⁵, a mean value for $k_{g(H_2O)}$, applicable to the sea surface, of about $3,000 \text{ cm h}^{-1}$, may be derived. This figure, along with appropriate values for the Henry's law constant, can be substituted into equations such as (8) and (9) in order to split observed values of $K(=1/R_l)$ into their liquid and gas phase components.

Liquid phase resistance (r_l). A number of techniques have been used to determine K_l *in situ* at the sea surface. The results of these experiments have been summarised by Liss⁴ and Broecker and Peng⁶ and a mean value of $K_l=20 \text{ cm h}^{-1}$ seems reasonable.

For O_2 , $H \sim 30$, $k_{g(O_2)} \sim k_{g(H_2O)} = 3,000 \text{ cm h}^{-1}$. Substituting these, and a value for $K_l(O_2)$ of 20 cm h^{-1} into equation (9), shows that $r_l \gg r_g$ and so $k_{l(O_2)} = 20 \text{ cm h}^{-1}$, that is, for O_2 all resistance to transfer across the interface occurs in the liquid phase. The same is true for all sparingly soluble gases which are not chemically reactive, for example N_2 , and the noble gases.

In the case of a chemically reactive gas, transport across the liquid film may be more complex than by straightforward

$$\alpha = [\tau(\sqrt{(K_0^1 \tau/D)} \cdot z_1)] / [(\tau-1)(\sqrt{(K_0^1 \tau/D)} \cdot z_1) + \tanh(\sqrt{(K_0^1 \tau/D)} \cdot z_1)] \quad (11)$$

where α is the fractional increase in the exchange constant due to chemical reactivity; τ the ratio of total to ionic forms of inorganic carbon; K_0^1 the hydration rate constant for CO_2 in water; and z_1 the thickness of liquid film.

The degree of exchange enhancement for CO_2 depends on the pH (which determines τ), the thickness of the interfacial liquid layer and the hydration rate constant. It has been shown in laboratory experiments that K_l increases approximately as the wind velocity squared^{4,8,9}, and that enhancement of $K_l(CO_2)$ over $K_l(O_2)$ can occur at low wind speed⁴. Under more turbulent conditions the thickness of the liquid film decreases to such an extent that re-equilibration of ionic species becomes much less important and is overridden by molecular diffusion. This is the situation at the sea surface, where conditions are generally too rough for enhancement of CO_2 transfer by more than a few per cent^{4,7,10}. In the case of gases with larger hydration rate constants, however, such enhancement may become much more important (see SO_2 later).

We have so far attempted to establish that values for the gas and liquid phase exchange constants appropriate to the sea surface are

$$k_{g(H_2O)} = 3,000 \text{ cm h}^{-1}; k_l = 20 \text{ cm h}^{-1}$$

In order to obtain the k_g value for gases other than water, the value of $k_{g(H_2O)}$ must be multiplied by the ratio of the square roots of the molecular weights of H_2O and the other gas. The value for k_l is based largely on measurements of CO_2 exchange and is probably valid for gases of molecular weight 40 ± 25 . For gases outside this range k_l should be multiplied by the ratio of the square roots of the molecular weights of CO_2 and the other gas. For a chemically reactive gas it is probable that k_l may be corrected for chemical enhancement

Table 1 Calculation of Overall Exchange Constants for a Number of Gases crossing the Air-Sea Interface

Gas	k_g (cm h^{-1})*	α †	k_l (cm h^{-1})‡	H	r_g (h cm^{-1})	r_l (h cm^{-1})	r_g/r_l	K (cm h^{-1})§
SO_2	1,600	1.721	34,420	3.8×10^{-2} (ref. 11)	6.3×10^{-4}	1.1×10^{-6}	573	1.6×10^3 (g)
N_2O	1,900	1.0	20	1.6	3.3×10^{-4}	5.0×10^{-2}	6.6×10^{-3}	20 (l)
CO	2,400	1.0	20	50 (ref. 12)	8.3×10^{-6}	5.0×10^{-2}	1.7×10^{-4}	20 (l)
CH_4	3,180	1.0	20	42 (ref. 13)	7.5×10^{-6}	5.0×10^{-2}	1.5×10^{-4}	20 (l)
CCl_4	1,030	1.0	10.7	1.08¶	9.0×10^{-4}	9.3×10^{-2}	9.7×10^{-3}	10.7 (l)
CCl_3F	1,085	1.0	11.3	5¶	1.8×10^{-4}	8.8×10^{-2}	2.0×10^{-3}	11.3 (l)
MeI	1,070	1.0	11.1	0.24¶	3.9×10^{-3}	9.0×10^{-2}	4.3×10^{-2}	10.6 (l)
$(Me)_2S$	1,620	1.0	20	0.3 (ref. 14)	2.1×10^{-3}	5.0×10^{-2}	4.2×10^{-2}	19.2 (l)

* All values corrected for molecular weight.

† All gases, other than SO_2 , assumed to be unreactive ($\alpha=1.0$). α_{SO_2} calculated using equation (11) and $\tau-1$ (at pH 8.0) = 8.032×10^{-8} , $K_0^1 = 3.4 \times 10^6 \text{ s}^{-1}$ (ref. 15), $D = 1.8 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$, $z_1 = D(O_2)/k_l(O_2) = 3.96 \times 10^{-3} \text{ cm}$.

‡ Values for CCl_4 , CCl_3F and MeI corrected for molecular weight.

§ Overall exchange constant, $K=(r_g+r_l)^{-1}$, expressed on either a gas (g) or liquid (l) phase concentration basis.

¶ J. E. Lovelock, personal communication.

molecular diffusion. For example, with CO_2 as well as the usual concentration gradient for gas molecules in physical solution, at a suitable pH there is also a similar gradient for HCO_3^- and CO_3^{2-} ions. It has been shown in laboratory experiments^{4,7} that for $pH > 5$, and under moderately calm conditions, the ionic species gradient can contribute significantly to the exchange of CO_2 across the interface. Hoover and Berkshire⁷ have used a one-layer (liquid) film model to derive the following equation which successfully predicts the exchange enhancements for CO_2 found in laboratory experiments.

by multiplying it by the value of α derived using equation (11).

In Table 1 values of k_g and k_l , plus a knowledge of H , are used in conjunction with equations (8) and (9) to derive overall exchange constants (K_g or K_l) for each of a number of gases. In subsequent sections this value of K , together with the observed concentration difference of each gas across the air-sea interface, will be substituted into equation (2) to calculate the flux of that gas across the interface.

For all gases considered, other than SO_2 , liquid phase resistance controls the exchange ($r_l \gg r_g$). For SO_2 gas phase

resistance is all important ($r_g \gg r_l$). This is in part due to the high solubility of SO_2 , but also because of its extremely rapid hydration reaction. A fuller discussion of this point and values of the ratio r_g/r_l at pH values other than 8.0 may be found in refs 16 and 17. Exchange of other gases with high solubilities and/or rapid aqueous phase chemistry (for example, NH_3 , SO_3 and HCl) is probably also controlled by gas phase resistance.

Flux of Various Gases

Sulphur dioxide (SO_2). In order to substitute the value for $K_{\text{g}(\text{SO}_2)}$ given in Table 1 ($1.6 \times 10^3 \text{ cm h}^{-1} = 0.44 \text{ cm s}^{-1}$) in equation (2) the mean concentration of SO_2 in oceanic air is required. Junge¹⁸ found values in Hawaii and Florida of 1 and $3 \mu\text{g m}^{-3}$ respectively. As the measurements were made on the coast and the winds were predominantly from the sea, Junge concludes that these values are fairly representative of marine conditions. Of the measurements made at sea, those for the North Sea¹⁹ are in the range 3 to $9 \mu\text{g m}^{-3}$, and for the North Atlantic <1 to $4 \mu\text{g m}^{-3}$ (ref. 20). These observations seem to point to a value of about $3 \mu\text{g m}^{-3}$ ($3 \times 10^{-12} \text{ g cm}^{-3}$) for the mean SO_2 level in air over the oceans.

Because of the chemical reactivity of SO_2 (rapid hydration and oxidation), it seems reasonable to assume that the SO_2 concentration in oceanic surface waters is zero and that the oceans act as a perfect sink for SO_2 .

Substitution in equation (2) gives $F_{(\text{SO}_2)} = 4.2 \times 10^{-5} \text{ g cm}^{-2} \text{ yr}^{-1}$. If this flux occurs over the whole area of the oceans ($3.6 \times 10^{18} \text{ cm}^2$), the total flux of SO_2 from atmosphere to oceans is $1.5 \times 10^{14} \text{ g yr}^{-1}$. This figure is close to estimates of the total world input of SO_2 to the atmosphere from the burning of fossil fuels ($10^{14} \text{ g yr}^{-1}$, ref. 21).

There is good agreement between our value for the air-sea SO_2 flux and the estimates of Eriksson²² ($2 \times 10^{14} \text{ g yr}^{-1}$) and Robinson and Robbins²³ ($0.5 \times 10^{14} \text{ g yr}^{-1}$). Our estimate is substantially lower than that of Spedding²⁴ ($9.6 \times 10^{14} \text{ g yr}^{-1}$). This discrepancy arises from Spedding's use of a rather large value for $K_{\text{g}(\text{SO}_2)}$ (1.4 cm s^{-1}) and an atmospheric SO_2 concentration of $6 \mu\text{g m}^{-3}$.

Nitrous oxide (N_2O). Junge and Hahn²⁵, from measurements in the North Atlantic, found a mean N_2O air concentration of 0.25 p.p.m. (by volume) and an average concentration in the water (expressed on a gas phase basis) of 0.4 p.p.m. This gives a concentration difference of 0.15 p.p.m., which is equivalent to $0.09 \times 10^{-6} \text{ cm}^3 \text{ N}_2\text{O per cm}^3$ of water on a liquid phase concentration basis. Hence the concentration gradient driving the flux of N_2O from the water to the air is $1.8 \times 10^{-10} \text{ g cm}^{-3}$. Substitution of this concentration difference and $K_{\text{I}(\text{N}_2\text{O})} = 20 \text{ cm h}^{-1}$ in equation (2) gives $F_{(\text{N}_2\text{O})} = 3.2 \times 10^5 \text{ g cm}^{-2} \text{ yr}^{-1}$. Assuming this flux takes place all over the oceans, the total flux of N_2O from sea to atmosphere is $1.2 \times 10^{14} \text{ g yr}^{-1}$.

This sea to air flux of N_2O may be of very considerable importance in the oceanic nitrogen budget. The amount of fixed nitrogen entering the oceans from river inflow and rain is about $8 \times 10^{13} \text{ g yr}^{-1}$, of which approximately 10% ($9 \times 10^{12} \text{ g yr}^{-1}$) is removed to marine sediments in the form of organic nitrogen compounds²⁶. The flux of N_2O calculated above is equivalent to $7.6 \times 10^{13} \text{ g N yr}^{-1}$, which is large enough to account for all of the nitrogen inflow to the oceans not removed to the sediments.

Carbon monoxide (CO). From measurements in the Pacific and North Atlantic, Lamontagne *et al.*²⁷ gives a value of 0.13 p.p.m. for the marine aerial concentration of CO. This is in agreement with estimates by other authors which fall in the range 0.03 to 0.3 p.p.m. Concentrations in the water are subject to diurnal cycling, varying between 2×10^{-8} and $13 \times 10^{-8} \text{ cm}^3 \text{ CO per cm}^3$ of water²⁷. From measurements in the North Atlantic, Seiler (cited in ref. 28) obtains water values in the range 2×10^{-8} to 10×10^{-8} . We will use a mean

surface water concentration of 6×10^{-8} . The mean aerial concentration expressed on a liquid phase basis is $2.6 \times 10^{-9} \text{ cm}^3 \text{ CO per cm}^3$ of water, and so the concentration difference sea-air is 5.7×10^{-8} (that is, surface seawater is twenty-three times supersaturated). This is equivalent to $7.1 \times 10^{-11} \text{ g CO cm}^{-3}$. Substitution in equation (2), along with $K_{\text{I}(\text{CO})} = 20 \text{ cm h}^{-1}$, gives $F_{(\text{CO})} = 1.2 \times 10^{-5} \text{ g cm}^{-2} \text{ yr}^{-1}$. Provided the value used for the mean seawater concentration of CO is applicable to the surface oceans as a whole, the total flux of CO from sea to air is $4.3 \times 10^{13} \text{ g yr}^{-1}$. The magnitude of this calculated flux is about 20% of the amount of CO injected into the atmosphere by human activities ($2 \times 10^{14} \text{ g yr}^{-1}$) (ref. 28).

Methane (CH_4). The mean atmospheric concentration of CH_4 over the North Atlantic and North Pacific was found by Lamontagne *et al.*²⁷ to be 1.4 p.p.m. (by volume). This agrees well with other values found and estimated for the marine atmosphere which fall in the range 1.1 to 1.6 p.p.m. Lamontagne *et al.*²⁷ measured methane concentrations in surface seawaters at the same time as the air samples were taken, and obtained a mean water value of $4.0 \times 10^{-8} \text{ cm}^3 \text{ CH}_4 \text{ per cm}^3$ of water. Expressing the atmospheric CH_4 levels on a liquid phase basis, 1.4 p.p.m. = $3.3 \times 10^{-8} \text{ cm}^3 \text{ CH}_4 \text{ per cm}^3$ of water, and comparing this with the mean surface water CH_4 concentration of 4.0×10^{-8} , indicates that the water is only about 20% supersaturated with respect to CH_4 in the air. The concentration difference sea-air is $0.7 \times 10^{-8} \text{ cm}^3 \text{ CH}_4 \text{ per cm}^3$ of water ($= 5.0 \times 10^{-12} \text{ g CH}_4 \text{ per cm}^3$ of water). Substitution in equation (2), along with $K_{\text{I}(\text{CH}_4)} = 20 \text{ cm h}^{-1}$ gives $F_{(\text{CH}_4)} = 8.8 \times 10^{-7} \text{ g cm}^{-2} \text{ yr}^{-1}$. If this flux occurs over the whole of the ocean surface, the total flux of CH_4 from sea to air is $3.2 \times 10^{12} \text{ g yr}^{-1}$.

The input of methane from the ocean to the atmosphere is more than an order of magnitude less than the flux of carbon monoxide calculated in the previous section. This arises because of the rather small degree of supersaturation of CH_4 at the ocean surface.

Carbon tetrachloride (CCl_4). Lovelock *et al.*²⁹ found a mean concentration of CCl_4 in the marine atmosphere over the Atlantic of 71.2×10^{-6} p.p.m. (by volume). Expressed on a liquid phase basis this is $66 \times 10^{-12} \text{ cm}^3 \text{ CCl}_4 \text{ cm}^{-3}$. The mean surface water concentration found for samples collected at the same time as the air samples was $60 \times 10^{-12} \text{ cm}^3 \text{ CCl}_4 \text{ cm}^{-3}$. Thus the air-sea concentration difference is $6 \times 10^{-12} \text{ cm}^3 \text{ CCl}_4 \text{ cm}^{-3}$, which corresponds to $4.1 \times 10^{-14} \text{ g CCl}_4 \text{ cm}^{-3}$. Substitution in equation (2) along with $K_{\text{I}(\text{CCl}_4)} = 10.7 \text{ cm h}^{-1}$ gives $F_{(\text{CCl}_4)} = 3.9 \times 10^{-9} \text{ g cm}^{-2} \text{ yr}^{-1}$. The total flux of CCl_4 from air to sea is $1.4 \times 10^{10} \text{ g yr}^{-1}$.

The total industrial production of carbon tetrachloride is about $10^{12} \text{ g yr}^{-1}$, of which about 95% is converted to CCl_3F and similar compounds. Hence the maximum amount of CCl_4 which can escape to the air is $5 \times 10^{10} \text{ g yr}^{-1}$ (ref. 29). Our calculated total flux of CCl_4 from the atmosphere to the oceans is about 30% of this figure.

Trichlorofluoromethane (Freon-11, CCl_3F). Lovelock *et al.*²⁹ have found a mean concentration of CCl_3F in the marine atmosphere over the Atlantic of 50×10^{-6} p.p.m. (by volume) or, expressed on a liquid phase basis, $10 \times 10^{-12} \text{ cm}^3 \text{ CCl}_3\text{F per cm}^3$ of water. The mean surface water concentration measured simultaneously was $7.6 \times 10^{-12} \text{ cm}^3 \text{ CCl}_3\text{F cm}^{-3}$. The concentration difference air-water = $2.4 \times 10^{-12} \text{ cm}^3 \text{ CCl}_3\text{F cm}^{-3} = 1.5 \times 10^{-14} \text{ g cm}^{-3}$. Substitution in equation (2) along with $K_{\text{I}(\text{CCl}_3\text{F})} = 11.3 \text{ cm h}^{-1}$ gives $F_{(\text{CCl}_3\text{F})} = 1.5 \times 10^{-9} \text{ g cm}^{-2} \text{ yr}^{-1}$. The air-sea flux of CCl_3F over the whole of the oceans is $5.4 \times 10^9 \text{ g yr}^{-1}$.

United States production of CCl_3F is $0.09 \times 10^{12} \text{ g yr}^{-1}$ (ref. 21). From this a total world production of about $0.3 \times 10^{12} \text{ g yr}^{-1}$ seems reasonable. As there are no known natural sources for this compound the calculated flux from the atmosphere to the ocean seems to be about 2% of the world production.

Iodomethane (MeI). Lovelock *et al.*²⁹ found a mean atmospheric concentration of iodomethane over the Atlantic of 1.2×10^{-6} p.p.m. (by volume). Expressed on a liquid phase basis this is 5.0×10^{-12} cm³ MeI per cm³ of water. The mean surface water concentration was 135×10^{-12} cm³ MeI cm⁻³. Hence the sea-air concentration difference = 130×10^{-12} cm³ MeI cm⁻³ = 8.2×10^{-13} g MeI cm⁻³. Substitution in equation (2) along with $K_{1(\text{MeI})} = 10.6$ cm h⁻¹ gives $F_{(\text{MeI})} = 7.6 \times 10^{-8}$ g cm⁻² yr⁻¹ and the total flux of MeI from oceans to atmosphere = 2.7×10^{11} g yr⁻¹.

It is well known that the I/Cl ratio in freshwater environments is much higher than the same ratio in seawater. One way to explain this is to assume that there is a preferential transfer of I relative to Cl across the sea-air interface. Miyake and Tsunogai³⁰ propose evaporation of I₂ molecules from the sea surface as the transfer mechanism. Such an explanation

only as good as the data used in the calculations. There is some uncertainty in choosing mean values for the gas and liquid phase exchange constants. For example it is not at all well known from measurements at the sea surface how k_1 varies with wind speed. Even greater uncertainties arise with the air and water concentrations for some of the gases. Many of these occur at low concentrations and require sophisticated apparatus for their detection. The problem is particularly acute for those gases where the degree of supersaturation is small. As further data become available it will be a relatively simple matter to recalculate the fluxes and to extend the approach to other gases. Although the model developed has been applied in this instance to the air-sea system, it should be possible to use it at any air-water interface in the environment provided the necessary data and field measurements are available.

Table 2 Calculated Fluxes of Various Gases crossing the Air-Sea Interface

Gas	SO ₂	N ₂ O	CO	CH ₄	CCl ₄	CCl ₃ F	MeI	(Me) ₂ S
Total oceanic flux g yr ⁻¹	1.5×10^{14}	1.2×10^{14}	4.3×10^{13}	3.2×10^{12}	1.4×10^{10}	5.4×10^9	2.7×10^{11}	7.2×10^{12}
	(-)	(+)	(+)	(+)	(-)	(-)	(+)	(+)

+, Sea → air; -, air → sea.

seems doubtful as no one has yet detected I₂ in seawater samples. Further, even if I₂ were found near the interface it would be likely to react with dissolved organic molecules which exist in high concentration at the sea surface³¹.

Miyake and Tsunogai³⁰ have calculated that in order to balance the iodine cycle on the Earth, 5×10^{11} g I yr⁻¹ must cross the sea-air interface. The calculation given above indicates that the flux of iodomethane from the oceans to the atmosphere is about 2.7×10^{11} g yr⁻¹, which is equivalent to 2.4×10^{11} g I yr⁻¹. This is approximately half the total amount of iodine needed to balance the budget for this element. In our view iodomethane is a much more likely vehicle than I₂ for the transfer of iodine from the ocean to the atmosphere.

Dimethyl sulphide (DMS, (Me)₂S). Lovelock *et al.*¹⁴ have found a mean surface water concentration of DMS in the Atlantic Ocean of 1.2×10^{-11} g cm⁻³. Dimethyl sulphide was not detectable in the marine atmosphere and has not yet been found in the open air. Taking the atmospheric concentration of DMS to be zero, the concentration difference between the sea and the air is 1.2×10^{-11} g cm⁻³. Substitution in equation (2) along with $K_{1(\text{DMS})} = 19.2$ cm h⁻¹ gives $F_{(\text{DMS})} = 2.0 \times 10^{-6}$ g cm⁻² yr⁻¹ and the total flux of DMS from oceans to atmosphere = 7.2×10^{12} g yr⁻¹.

In order to achieve balance, most budgets for sulphur at the Earth's surface have to invoke a process in which some volatile compound (usually H₂S) is produced over the land surface and/or the oceans. A recent sulphur budget³² gives a figure of 268×10^6 ton SO₄ yr⁻¹ (89×10^{12} g S yr⁻¹) for this flux.

The sea-air flux of dimethyl sulphide calculated above (7.2×10^{12} g yr⁻¹) is equivalent to 3.7×10^{12} g S yr⁻¹. This corresponds to about 4% of the amount needed to balance the sulphur budget. It would seem that if, as suggested by Lovelock *et al.*¹⁴, dimethyl sulphide is the "missing" volatile compound in the sulphur cycle then only a small fraction of it is released over the oceans, and a terrestrial source must be sought for the remainder.

Conclusions

The results of the flux calculations presented here are summarised in Table 2. Obviously the results presented are

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Temporal Variation of the Seismic Moment Tensor and the Evidence of Precursive Compression for Two Deep Earthquakes

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For two deep earthquakes, about 80 s before the abrupt onset of each, the hypocentral region begins to experience compression.

IN elastodynamic terms, an earthquake is caused by the sudden release of stress in the Earth. The difference in stress, before and after the earthquake, is termed the stress drop, and the volume integral of the stress drop, which has the dimensions of (force $\times$ distance), is termed the moment, or moment tensor. (In displaying the results of numerical calculations we shall use the sign convention consistent with the definitions of the tension and compression axes commonly used in seismological literature.)

The body forces, equivalent to the stress drop, are said to constitute the source mechanism of the earthquake. The balanced double-couple system of equivalent forces has been almost universally adopted in studies of the source mechanism of earthquakes; papers by Randall¹, Knopoff and Randall² and Randall and Knopoff³ represent notable exceptions. Representation of the stress drop by such a system involves two assumptions: (i) the stress is deviatoric; and (ii) the effective stress drop along one of the principal axes is equal to zero. If an earthquake should have, for example, a partly isotropic moment tensor, then studies of its source mechanism based upon the *a priori* assumption of a system of double couples must fail to reveal the isotropic part. It is clear that a procedure in which the elements of the moment tensor can be determined directly from the seismic data is preferable to one that imposes *a priori* restrictions.

It is also important that each of the elements of the moment tensor be independently determined as a function of time (or frequency). Such a general approach can be helpful in establishing the sequence of events at the source and provide data for testing the applicability of various dynamical source models.

New analysis technique

The general approach to the source mechanism problem has now become practically feasible because of the development of a novel method of processing the Fourier spectra of observed seismograms. The particular procedure applied here depends on the availability of digitised recordings from a large number of well distributed stations.

The theory of excitation of normal modes by point sources and the principle of retrieving the moment tensor from seismic data have been described by Gilbert^{4,5}. A finite duration (as opposed to instantaneous stress release) of a seismic event implies that the moment tensor is a complex function of frequency. We shall assume, however, that the source represents a point in space; possible errors due to this assumption are largely avoided by considering in the analysis only those modes with wavelengths that are approximately one order of magnitude greater than the expected source dimensions.

Because the temporal changes in the stress release pattern

are our principal interest, we shall determine in this report the moment rate tensor— $\dot{\mathbf{m}}(t)$, the time derivative of the moment tensor— $\mathbf{m}(t)$: $\dot{\mathbf{m}}(t) = d(\mathbf{m})/dt$. In the frequency domain, the relationship between the spectra of the moment rate tensor— $\dot{\mathbf{M}}(\omega)$, and moment tensor— $\mathbf{M}(\omega)$ is: $\dot{\mathbf{M}}(\omega) = i\omega\mathbf{M}(\omega)$. Consequently, the procedure described below can be used with only minor changes to determine the spectrum of the moment tensor.

Gilbert's result can be generalised for a source of finite duration; the amplitude of the ground motion due to a normal mode at a certain point on the surface of the Earth is a linear function of the elements of the moment rate tensor $m_j(t)$,

$$u(t, \theta, \phi) = \cos \omega_0 t \cdot \exp(-\omega_0 t/2Q) * \sum_{j=1}^6 a_j m_j(t)$$

where the symbol $*$ denotes convolution and where the coefficients a_j depend on coordinates θ and ϕ of the receiver, the location of the source and the assumed model of the Earth; parameters ω_0 and Q represent the eigenfrequency and the quality factor of a particular mode. The dependence of u on ω_0 and Q is eliminated by calculating the Fourier transform of the equation above and integrating the resulting cosine and sine transforms over the entire frequency range. Having made observations at a large number of well distributed stations we can solve a system of normal equations for the components of $\dot{\mathbf{M}}_j(\omega_0)$, the Fourier transform of $m_j(t)$ at ω_0 .

In reality, the observed spectra represent the superposition of a great many modes and the method cannot be applied as described above. But the spectral energy of a mode is strongly concentrated about its eigenfrequency, and it is sufficient to perform the integration in the frequency domain over a relatively narrow frequency interval in order to retrieve nearly all of its energy. This still leaves open the possibility of interference of another well excited mode with an eigenfrequency close to that of the currently analysed one. But, if the receiver coverage is sufficiently dense, the effect of the modes with angular order numbers different from that of the mode of interest is largely eliminated from the final system of normal equations. The elimination would be complete if the coverage were continuous, because of the orthogonality of the spherical harmonics which enter into the coefficients a_j . This is the same principle which enabled Mendiguren^{6,7} and Dziewonski and Gilbert⁸ to isolate spectral peaks of normal modes which could not be recognised in the spectra of individual recordings.

Application of the method

Because of the integration procedure described above, the success of the method depends to a large extent upon the precise knowledge of the eigenfrequency of each normal mode for which we attempt to determine the moment tensor. Our previous observations of normal modes⁸⁻¹⁰ and inversion studies^{9,11,12} have been very helpful in this respect.

Because a moment rate tensor can be expected to vary rather slowly with frequency, we have chosen in this study to determine an average moment rate tensor for a number of

adjacent and equal frequency intervals by taking into account excitation of all modes with eigenfrequencies within a prescribed interval and wavelengths greater than an assumed minimum.

The principal advantage of the method proposed here is that in studying the source mechanism we can utilise the entire information contained in a seismic recording. Consequently, the moment rate tensor is derived from the energy of modes representing a wide range of phase velocities, wavelengths and various ratios of compressional and shear energies^{9,10}. All previous attempts to determine the source mechanism from the observed amplitudes of surface waves or free oscillations were confined to the fundamental modes. There are, of course, practical limitations on the frequency range of the proposed analysis. The magnitude of an earthquake combined with the instrumental response of the recording stations determines the lower frequency limit. The present upper frequency limit (about 0.014 Hz) is imposed only by the extent of the existing theoretical calculations of eigenfrequencies and eigenfunctions of normal modes.

We have applied the theory and method described above to the analysis of the stress release process of two deep South American earthquakes: the Colombian earthquake of July 31, 1970 (1708 : 05.4 GMT, 1.5° S, 72.6° W, $h=650$ km) and the Peru-Bolivia border earthquake of August 15, 1963 (1725 : 27.9 GMT, 13.8° S, 69.3° W, $h=543$ km).

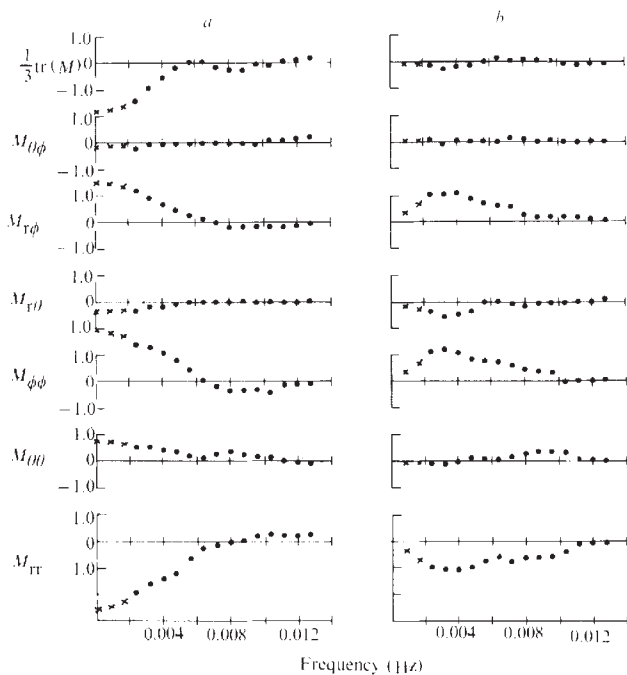


Fig. 1 Moment rate tensor of the Colombian earthquake of July 31, 1970, as a function of frequency. *a*, Real; *b*, imaginary. ●, Values determined from the analysis of excitation of normal modes; ×, extrapolated values. The isotropic component of the moment rate tensor is shown at the top; the remaining diagrams represent the deviatoric part of the moment rate tensor; that is, the isotropic stress rate has been removed for the measured values of the elements M_{rr} , $M_{\theta\theta}$ and $M_{\phi\phi}$. The coordinate system adopted is that of the lower hemisphere with the r axis pointing downwards, θ axis to the north and ϕ axis to the west. To obtain a result in (dyne-cm) the values shown in the figure should be multiplied by a factor of 5×10^{27} .

Figure 1 shows the result of processing 165 recordings of the Colombian earthquake. The centre frequencies of individual intervals were $(1/2\pi) \cdot 0.005 n$ Hz ($n=3, 4, \dots, 16$) and the half width was $(1/2\pi) \cdot 0.0025$ Hz. The total number of modes considered was 1,348 (only those modes with wave-

lengths greater than 1,000 km were taken into account) for a total number of observational equations of over 200,000. The number of observational equations for individual frequency intervals was variable but never less than 5,000. Error estimates were obtained from regression analyses but they are too small to be shown in the scale of the figure.

The isotropic stress rate is shown at the top of the figure (negative stress corresponds to compression), and the remaining diagrams represent the elements of the deviatoric part of the moment rate tensor; that is, the isotropic stress rate has been subtracted from the diagonal elements M_{rr} , $M_{\theta\theta}$ and $M_{\phi\phi}$.

If the stress release were instantaneous (a step function in time), the imaginary part of the moment rate tensor should be equal to zero, and the real part should be independent of frequency. This is clearly not the case. If the stress were released uniformly over a period of time from $t=0$ to $t=t_1$, then the cosine transform should be proportional to $\sin \omega t_1 / \omega t_1$ and the sine transform to $\sin^2(\omega t_1/2) / (\omega t_1/2)$. With the exception of the isotropic component, this seems to be a relatively good approximation, and the least squares fit yields $t_1 = 78$ s.

The isotropic component shows an entirely different behaviour, with the imaginary component being nearly zero at all frequencies and a significant real component decreasing in frequency more rapidly than all the other elements. This implies a non-zero isotropic stress rate at negative times, or, in other words, a compression preceding the origin time determined from the first arrivals of body waves.

The results of the analysis of 46 digitised recordings of the

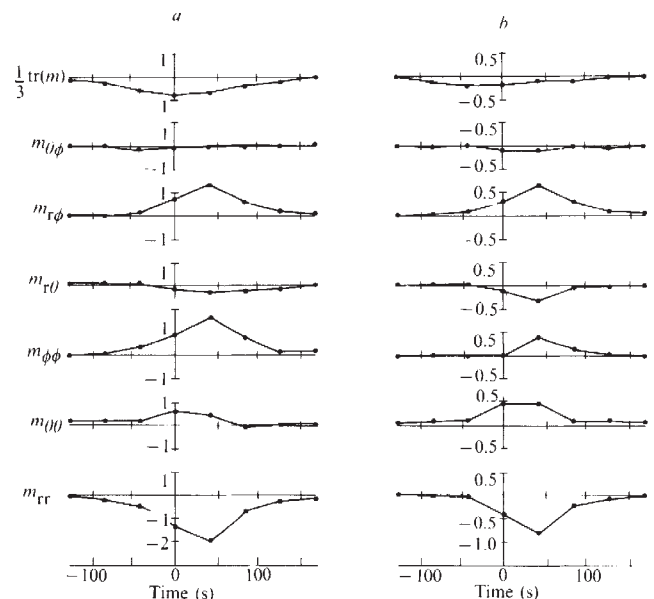


Fig. 2 Moment rate tensors of the Colombian earthquake of July 31, 1970 (*a*) and Peru-Bolivia border earthquake of August 15, 1963 (*b*) as a function of time. Significance of the components of the moment rate tensor is the same as in Fig. 1. To obtain a result in (dyne-cm/sec) the values shown in the figure should be multiplied by a factor of 4×10^{25} .

Peru-Bolivia border earthquake also indicate a release of purely compressional stress at negative times. The elements of the moment-rate tensor for this earthquake are 2.5 times less, on average, than for the Colombian event and the estimate of the uniform radiation time is also lower: $t_1 = 72$ s.

Our results must be extrapolated for the low frequencies ($n=0, 1$ and 2) before the frequency domain data can be synthesised into the time domain. Such a procedure is always somewhat arbitrary, but on the basis of theoretical considerations of Aki¹³ and the experimental results of Mendiguren⁶

for the Colombian earthquake we felt that we were justified in extrapolating the moment rate tensor obtained by the least squares fit of a uniform radiation source. The crosses in Fig. 1 identify values obtained by extrapolation; these values appear to merge smoothly with the experimental data. We believe that the results described later are not materially affected by the uncertainty that might have been introduced by extrapolation.

The time functions of the moment rate tensor are shown in Fig. 2 (it should be remembered that the values shown in the figure do not refer to an instant of time $t = n\Delta t$ ($\Delta t \approx 40$ s) but they represent an average stress release over a period of time from $(n-1)\Delta t$ to $(n+1)\Delta t$). The moment rate tensor decays to a zero value rather rapidly for both positive and negative times, which indicates that the long-period noise is low. The compressional isotropic stress-rate represents a dominating factor at negative times, but there also seems to be a detectable amount of shear stress released during the last 80 s interval before the 'origin time'.

In the next step, we find the principal stress rates and directions of the principal axes of the moment rate tensor for a number of time intervals. The results are shown in Fig. 3, where the axes are numbered according to the decreasing absolute value of the principal stress rate. The directions of the axes are shown only for $t = -40, 0, 40$ and 80 s, because at other times the shear stress rate is comparable to the noise level and the results are incoherent.

The total stress change can be found by integration in time of the results shown in Figs 2 and 3. For example, we have computed the total change in the isotropic stress to be -5.1×10^{27} dyne-cm for the Colombian event and -1.3×10^{27} dyne-cm for the Peru-Bolivia earthquake; using the elastic constants of the model B497¹⁰, we find the decrease of volume to be 1.9 and 0.5 km³, respectively.

Comparison with other work

Our results are compared with the directions of the compression, tension and null axes found from the fault plane solutions by Mendiguren⁶ for the Colombian earthquake and Chandra¹⁴ for the Peru-Bolivia border event.

Our solution is fairly consistent with that of Mendiguren, except for a horizontal rotation of all three axes by approximately 20° and a slow decrease of the plunge of the dominating axis of compression from 79° to 65°.

The differences with the solution of Chandra are much more significant. It seems that his determination of the direction of the tension axis has been in error; it remains at odds with all the other results from this region¹⁵. Our results show that the azimuth of the tension axis of the average stress released between 0 and 80 s after the origin time is 232°, which is in a good agreement with the trends found from other fault plane solutions by Isacks and Molnar¹⁵. The most striking result of our analysis of the Peru-Bolivia border earthquake is the significant rotation in time of axes II and III, whereas the position of axis I remains almost unchanged at positive times. This observation indicates that in some cases the pattern of stress release may undergo a significant change during the event.

The validity of a solution for a moment tensor can be confirmed by a theoretical calculation of the excitation of normal modes and comparison of this result with the observed spectra. The method of phase equalising the observed spectra for spectral peaks belonging to individual normal modes represents a severe test of a given source mechanism, since a successful result of this operation depends on the ability to estimate correctly the amplitudes of all the modes within a certain frequency band. We have performed such calculations for the Colombian earthquake data using both the double couple solution of Mendiguren⁶ and our results shown in

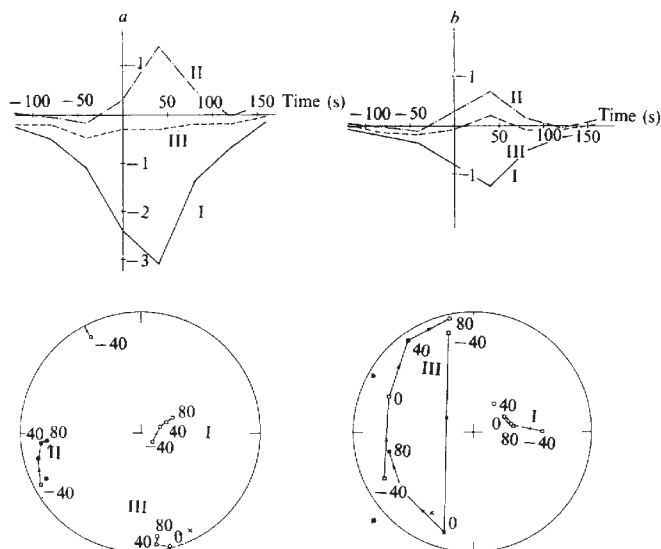


Fig. 3 Temporal variations of the principal stress rates and directions of the principal axes of the moment rate tensor (*a* and *b* and the scale conversion factor as Fig. 2). The large circles in the lower part of the figure represent equal area projections of the lower hemisphere of a focal sphere. Results obtained from fault plane solutions by Mendiguren³ (left) and Chandra¹⁴ (right) are shown for comparison. ○, ●, ×, compression, tension and null axes from fault plane solutions.

Fig. 1. The results obtained using our solution were generally better than when the double couple model was used. Improvement was especially significant for the modes with a large content of compressional energy and relatively long periods ($T > 200$ s); it can be shown that these modes are particularly sensitive to the isotropic component of a moment tensor. The outcome was also successful when we used our solution for the Peru-Bolivia earthquake, but we have not tested the solution of Chandra. In general, the results of the test confirm the consistency of our moment tensors with the observations.

Our results show that a hypocentral compression precedes the main phase of both earthquakes analysed here. This effect may be caused by a transition of the matter in the mantle into a denser phase. If such a change is associated with a release of thermal energy, it might provide an increase in temperature which is necessary to initiate a shear-melting instability which has been suggested by Griggs and Baker¹⁶ as a possible cause of deep earthquakes.

The results in Fig. 3 indicate that some shear stress is also released prior to the 'origin time'. The directions of the principal axes of stress rate at negative times are basically consistent with those determined for the main phases of the earthquakes. The statistical significance of this result is less certain than in the case of isotropic stress, because of the difference of magnitude in the deviatoric part of the moment rate tensor before and after the origin time. But if this result is correct it further confirms the theory of Griggs and Baker which predicts the phase of a relatively slow release of shear stress before the occurrence of shear melting.

Further possibilities

Our results for the two earthquakes seem to confirm that, if the data are adequate, fault-plane solutions provide a reasonably good estimate of the directions and signs of the principal axes of a moment tensor. However, the magnitude of the deviatoric stress rate estimated for axis III represents approximately one-fifth of that of axis I and it is statistically significant. This indicates that although the double couple model may represent a convenient approximation, the mechan-

ism named by Knopoff and Randall² a 'linear compensated vector-dipole' could possibly play a significant role in the process of stress release at long periods.

It is not justified to extrapolate conclusions made on the basis of observations in one frequency range into an entirely different frequency range. For example, the temporal variations of the release of isotropic stress indicate that it is a long-period phenomenon; the rate of its release does not appear to change at the 'origin time' (Fig. 2). Consequently, one should not expect to observe a significant effect of a volume change on the amplitudes and signs of the first arrivals of body waves. It would be wrong, of course, to infer from the short period data that a change of volume did not take place, yet such inferences have been made in a number of published papers.

The sequence of events at the source observed for the two analysed earthquakes leads us to the following speculation. If the volume change which preceded the release of the short period energy were insufficient to initiate the shear melting instability, then such a 'silent earthquake' would most likely remain unreported. It may be that hypocentral volume changes which are not followed by the release of short period energy occur frequently. Perhaps the largest of those might have generated free oscillations of the Earth that could be detected by very sensitive instruments.

We believe that analyses such as presented here should be applied to recordings of other events from different tectonic regions and different depth ranges. Information on the stress release prior to the main phase of earthquakes may provide important clues with respect to the problem of earthquake prediction.

Our method of studying the source mechanism can be extended to travelling waves and used to study the source

properties of smaller events. In particular, determination of the ratio of energies released by the isotropic and deviatoric part of the moment tensor may be useful as a discriminant between natural events and explosions¹⁷.

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Structural Response of Vertebrate Photoreceptor Membranes to Light

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A distinct structural response in disk membranes from frog retinal rod outer segments was found directly proportional to exposure to light. Translocation of rhodopsin from the intradisk hydrophilic surface to the internal hydrophobic phase of the membrane was observed.

ABSORPTION spectral changes following illumination of the chromolipoprotein rhodopsin in the stack of laminar disk membranes of vertebrate rod photoreceptor cells have generally been interpreted as conformational changes in the lipoprotein moiety¹⁻⁴. Although optical rotatory dispersion and circular dichroism studies of rhodopsin do not seem to support this idea^{5,6}, it is difficult to arrive at any other molecular interpretation, particularly in view of the established pH changes and the exposure of titratable thiol groups that accompany certain of these intermediate processes^{3,4}.

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The question naturally arises as to whether such suspected molecular changes are reflected on the gross structural level of the membrane itself. A substantial amount of rhodopsin⁷ in the photoreceptor disk membrane of the frog coupled with the evidence of light-induced conformational changes in the rhodopsin moiety¹⁻⁴ indicate that a membrane structural response to light may occur.

Studies of orientated hydrated pellets of isolated disk membranes by X-ray diffraction⁸ indicate that structural changes in the disk membranes do occur upon illumination and a slight movement of rhodopsin in the disk membrane upon light exposure has been detected by Blaisie^{9,10}. Furthermore, studies of the thermal broadening of X-ray diffraction maxima and the lipid and fatty acid composition of the membrane^{11,12} indicate a fluid-like milieu, which makes possible mobility for the chromophore in the disk photoreceptor membrane. In the light of these indications we have attempted to visualise, in a direct manner, such structural changes that might occur in the photoreceptor membrane upon light exposure.

Here we describe the results of our application of freeze-cleave-etch electron microscopy to study photodynamic responses in intact retina, rod outer segments, and disk membranes of the frog, *Rana catesbiana*. The characteristically weak intermolecular interactions in the hydrophobic portion

of the disk membrane and the relatively strong interaction of hydrophilic protein with surrounding cell stroma enable this technique to discriminate among three membrane surfaces: the external hydrophilic surface of the disk, the interior hydrophobic region of the disk bilayer, and the internal hydrophilic disk surface, enclosing an internal aqueous region. By electron microscopy of cleaved, shadowed and etched samples, a distribution of these three surfaces can be seen.

Several preparations of the retina from dark adapted frogs were examined in isotonic Boyle-Conway medium¹⁸, as well as in hypertonic and hypotonic solution. Intact rods were prepared by dissection of whole retina into the Boyle-Conway medium and either flotation of outer segments in 50% buffered sucrose or agitation of the retina and filtration through nylon netting. In addition, pure rods were prepared by density gradient centrifugation and examined. Whole retinæ were gently excised and frozen in Freon 22 on stainless steel planchets; these were immediately subjected to a high vacuum (2×10^{-6} torr) at -196°C , in a Denton Co. freeze-etch apparatus. Additional samples were frozen either in the dark or after various stages of light exposure up to 2 min at 25°C . The apparatus containing the planchets was evacuated, the samples cleaved at -197°C and subsequently etched at 95°C for 2 min. The etched samples were then replicated with platinum-carbon, floated on water and taken up on carbon coated copper grids. All electron microscopy was performed with a Hitachi 11-UA instrument.

Membrane surfaces

By this technique we have made many observations on the photoreceptor disk membranes of the frog and have found large differences between dark and illuminated membrane surfaces. In the dark (Fig. 1), all cleaved disk membranes examined showed a smooth outer hydrophilic surface, a particle-free rippled hydrophobic surface and a highly particulated intradisk hydrophilic surface. Identification of

these surfaces was made on several bases. First, samples were subjected to etching without previous cleaving. By this technique, it could be assumed that exclusively external disk surfaces were observed. Second, membranes were subjected to light sonication for 10 to 15 s and subsequently etched without previous cleavage. In dark-adapted sonicated samples, only a smooth and particulated surface was observed. The surface bearing particles was similar to the internal hydrophilic surface observed by the cleave-etch technique with dark-adapted membranes. Upon similar sonication treatment of light-adapted samples, only smooth surfaces were observed, implying that translocation of the particles away from the intradisk hydrophilic surface had occurred. Third, a morphologically different surface was evident upon cleavage of the sonicated preparation prior to etching. This surface was the preferred cleavage plane and exhibited a 'rippled' texture. We believe this surface to be the hydrophobic region of the membrane.

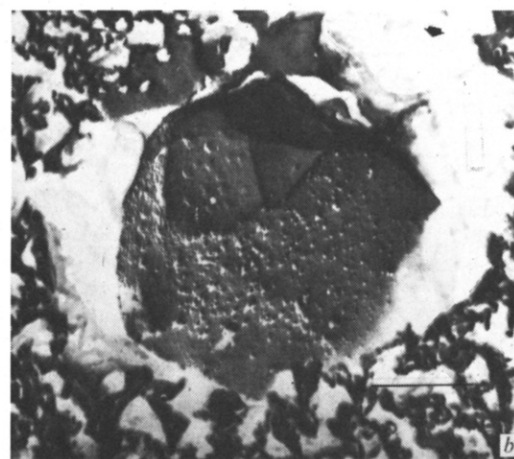
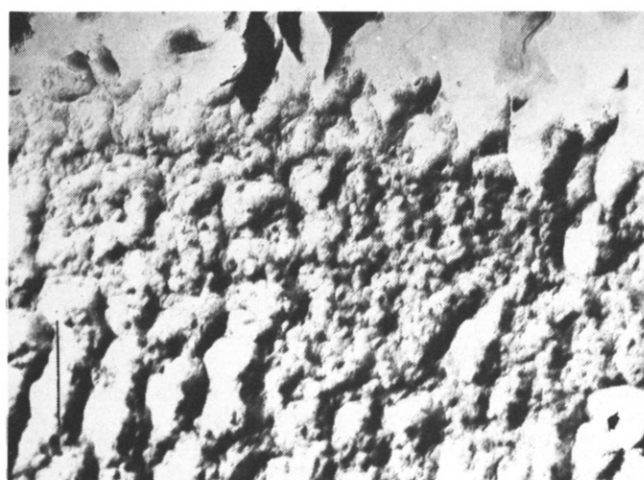


Fig. 2 *a*, Fully bleached disk membrane fragment from preparation of isolated *R. catesbeiana* rods; note particulated hydrophobic surface with ~ 120 – 150 Å particles (bar = 0.32 μm). *b*, Fully bleached disk membrane fragment from *R. catesbeiana* rods; note particulated hydrophobic surface and adjacent smooth hydrophilic surface (bar = 0.12 μm). Arrows indicate direction of platinum shadow.

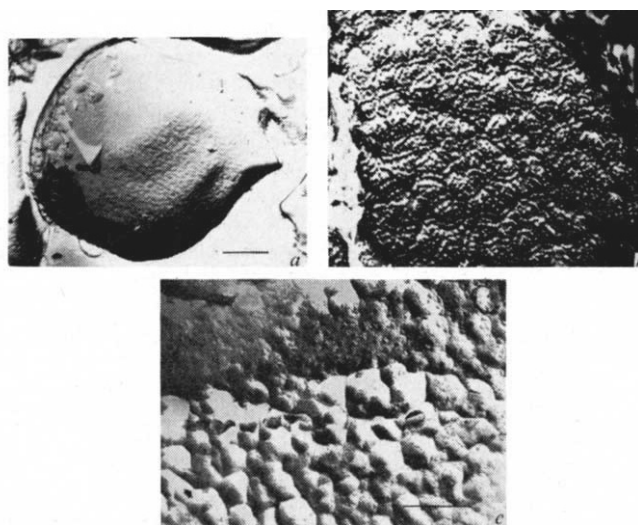


Fig. 1 *a*, Partial surface of dark-adapted photoreceptor disk membrane from preparation of isolated *Rana catesbeiana* rod outer segments; note particle-free outer hydrophilic and rippled hydrophobic surface (bar = 0.8 μm). *b*, Partial surface of dark-adapted photoreceptor disk membranes from preparation of isolated *R. catesbeiana* rods; note linear arrays of ~ 50 to 60 Å particles on intradisk hydrophilic surface (bar = 0.18 μm). *c*, Dark-adapted photoreceptor disk membrane fragment from preparation of *R. catesbeiana* rods; note particle-free, rippled hydrophobic region in lower half of figure, and particulated surface immediately above. A smooth membrane area representing an external hydrophilic surface is seen on the upper left hand portion of the figure (bar = 0.10 μm). Arrows indicate direction of platinum shadow.

The particles on the internal hydrophilic surface of the dark-adapted preparations, tentatively identified as rhodopsin, were of the order of 50 Å diameter and seemed to project more than 20 Å beyond the internal hydrophilic membrane surface, although it was difficult to determine the exact

height of particles by shadow length in freeze-etched preparations because of random sample tilt in the plane of the incident shadow. Further examination showed the particles to be discrete with a distinct, short-term order relative to other particles, that is, protuberances were arranged in a curvilinear array of ten to twenty discrete particles as seen in Fig. 1*b*. This ordering is not inconsistent with the planar square array reported by Blasie *et al.* on the basis of an optical transform of electron micrographs of isolated disk photoreceptors¹⁴.

After light exposure at room temperature for 2 min at a distance of 4 foot from a standard 150 W bulb to yield predominantly metarhodopsin₃₈₀II in the disk membrane (by spectral monitoring) and quick freezing of the samples, a substantial change in the macroscopic surface structure of the disk membrane was observed (Fig. 2). Once again, a smooth outer hydrophilic surface was apparent. This surface was clearly identified in uncleaved etched samples of disk membranes and showed no change on light exposure. The internal hydrophilic region, however, was now smooth and showed no particulate structure while the inner rippled hydrophobic phase was observed to have a high density of particles of about 125 to 175 Å in diameter projecting 20 to 30 Å above the cleavage surface. In contrast to those seen on the interior hydrophilic surface before illumination, these particles showed no long or short term order and were randomly distributed on the membrane surface. Furthermore, the particles protruding from the hydrophobic surface after light exposure were arranged in what appeared to be aggregates of four to eight molecules and the average distance between centres in the hydrophobic region was smaller than that between particles arrayed on the internal hydrophilic surface of dark-adapted disk membranes.

Rhodopsin molecules

Identification of the observed particles as rhodopsin molecules is based on several points of evidence. The first is the very high density of particles on the membrane surface before and after light exposure, that is, one molecule per 5,400 Å². Other known molecular species would not be expected to occur in the disk photoreceptor at this membrane density. The particle density obtained is consistent with that calculated indirectly by X-ray diffraction¹⁵ and microspectrophotometric studies on rhodopsin-containing disk membranes¹⁶.

A second point of evidence is based on observations of membranes from which rhodopsin is extracted by detergent. Lightly sonicated (15 s with a Branson model JI7A sonicator, tuned to 20 kHz, yield of 250 W (acoustical)) disk membranes were extracted in the dark with 3×10^{-4} M aqueous digitonin for 30 min and then centrifuged. The absorbances at 500 nm of the resulting supernatant were taken and the pellet studied by freeze-cleave-etch electron microscopy. It was found that the amount of rhodopsin extracted increases from an initial ΔA_{500} of 0.004 for the first extraction to 0.467 for the third extraction. Electron micrographs of a pellet frozen in the dark after the first extraction revealed the same particle density on the internal hydrophilic disk membrane as untreated membranes with some slight membrane disruption. After the second extraction the number of molecules on the inner hydrophilic surface had markedly decreased to a particle density of one molecule per 14,000 Å². The third digitonin treatment disrupts the membrane appreciably, but there is a noticeable decrease in the particle density although it is difficult to quantify.

A third point of evidence derives from the fact that when membranes are fixed in 2% buffered glutaraldehyde in the dark, subsequent light exposure does not induce translocation of the particles to the hydrophobic phase, and the same 50 Å particles remain in linear arrays on the intradisk hydrophilic surface. This observation indicates that the integrity of the lipoprotein must be maintained if the translocation

mechanism is to take place. These three points of evidence, though indirect, clearly suggest that the particles seen on the inner membrane are rhodopsin molecules.

On the basis of the observations reported here the native disk photoreceptor membrane seems to be a highly asymmetric protein-lipid bilayer with rhodopsin bound only on the internal disk membrane surface. When the outer hydrophilic membrane surface is visualised with the present technique, it appears, in many cases, to reflect the rippled texture of the hydrophobic phase beneath. On the other hand, no rippling is observed on the inner hydrophilic membrane surface after light adaptation, indicating that this surface is considerably more dense than the outer. These observations are in harmony with the X-ray diffraction studies of Worthington¹⁵ which also suggest an asymmetry of electron density in the disk membranes.

In addition to the above studies, whole intact retina was also subjected to similar treatment. Retinae were studied as fully dark- or light-adapted tissue and in all cases exhibited behaviour identical to the isolated rods and disk membranes. Furthermore, rod outer segments subjected to hypertonic or hypotonic buffer treatment showed respectively shrinking or swelling of the intradisk space, but no deviation in the translocation behaviour within the membrane was observed as a result of this treatment.

Particle translocation

The degree of particle (rhodopsin) translocation is highly dependent on the extent of photobleaching (Fig. 2). Preparations of frog rod photoreceptors were photolysed for 0, 10, 30, 40, 80, 90 and 120 s and samples taken at each bleaching level for freeze-etch electron microscopy. Samples were also taken at each level of bleaching for extraction with 1% Emulphogene in 0.1 M hydroxylamine at pH 7.2 and a ΔA_{500} measurement made upon photobleaching of the extracted material. It was observed that the number of particles which were translocated from the internal hydrophilic disk surface to the hydrophobic region of the disk was directly proportional to the extent of photobleaching as measured by spectral ratio. This is further evidence that the particles that translocate in the disk membrane are indeed rhodopsin. In partially bleached samples, the A_{500} absorbance difference at 10 s illumination was 0.59 and at 90 s was 0.018, with the particle density on the hydrophobic surface changing from 15 per 25×10^6 Å² at 10 s to 125 per 25×10^6 Å² at 90 s (Table 1). When full bleaching occurred,

Table 1 Absorbance and Particle Density Differences of Frog Rod Photoreceptors Differentially Bleached

Bleaching time (s) *	ΔA_{500}	Particle density † per 25×10^6 Å ²
0	0.068	5
10	0.059	15
30	0.045	35
40	0.042	50
80	0.028	90
90	0.018	125
120	0.008	135

* Exposed at 25° C at a distance of 4 foot from a standard 150 W light bulb with constant agitation of the sample.

† No. on median hydrophobic surface.

a particle density on the hydrophilic surface was not wholly detectable or possible to quantify. Concomitant with this result, the ΔA_{500} was nearly zero.

The experimental results described above are consistent with the view that the rhodopsin molecules are located on the internal, intradisk hydrophilic surface of the disk membrane. Illumination in conditions terminal at metarhodopsin II seems to initiate a translocation of the rhodopsin mole-

cules to the interior hydrophobic region of the membranes with their subsequent aggregation. This apparent translocation is consistent with a change in the lipoprotein configuration of rhodopsin, manifest as a change in surface properties from a charged hydrophilic to an uncharged hydrophobic character. Such a process, clearly, could quite plausibly be associated with release of a transmitter substance such as calcium ion from the membrane. We stress, however, that although the evidence supporting the location of the rhodopsin molecules on the internal hydrophilic surface of the disk membrane and their translocation to the median hydrophobic region on illumination is highly indicative, it is not at this stage unequivocal. Furthermore, it should be pointed out that the light levels employed in this study are far from physiological. Normally one might expect at most a few molecules in a given membrane to be bleached within the refractory period of the rod; whether the changes would be qualitatively the same in these conditions as under the conditions of the experiment is a moot point.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Volcanic Couples and Deep Mantle Plumes

MORGAN¹ has convincingly demonstrated a case for the upward rise of deep mantle material along narrow plumes which give rise to positive gravity anomalies and hot spots on the Earth's surface. These hot spots are reflected in large volcanoes and, as Wilson² suggested, where the lithosphere moves over hot spots, volcanic chains are produced. Perhaps the most striking support of Morgan's hypothesis are the parallel 'dog legs' in hot spot trajectories given by the Hawaii-Emperor, Austral-Marshall/Gilbert, and Tuamotu-Line chains, most readily explicable as generated by motion of the Pacific plate over three fixed hot spots.

Abundant support for the mantle plume hypothesis is becoming available from a number of approaches³⁻⁶. Gass³, Burke and Wilson⁴, and LeBas⁵ have emphasised that African rifts can be explained by propagation of cracks produced by doming of the African craton, domes which they interpreted as resulting from upwelling mantle plumes. Schilling⁷ has explained trace element and isotope variations of Iceland lavas on the basis of mantle plumes. Although this interpretation was criticised by O'Hara⁸, independent geophysical evidence⁹ also supports the concept of an Iceland mantle plume.

Although much geological, geophysical and geochemical evidence thus seems to be in favour of the hypothesis of deep mantle plumes, the ghost of Kelvin still walks among geophysicists and they have recently issued a statement of their disbelief¹⁰. Perhaps their disbelief is merely founded in semantics, and could be modified by an answer to Tozer's¹¹ 'cri de coeur' that someone "explain in terms that are meaningful to geophysicists in what respects the conventional geological pictures of rising magma differ from 'a thermal plume'." Although it is difficult to know what is meaningful to a geophysicist, the following observations on magma production may add some fuel to fire the geothermal plume debate.

Many large central volcanic edifices, both continental and oceanic, are formed from two (and rarely three or even four) volcanic centres spaced about 5 to 25 km apart. These I term 'volcanic couples', and several examples are given in Table 1. Note that many of these are shown as hot spots by Morgan¹.

Table 1 Comparison of Magma Types and Distances Separating Volcanic Centres of Particular Volcanic Edifices

Volcanic edifice (and reference)	Centre 1 basic magma type	Centre 2 basic magma type	Distance (km) between 1 and 2
Hawaii ¹²	Kilauea Tholeiite	Mauna Loa Tholeiite	30
K, Ba and so on enriched relative to M. Loa tholeiite			
Réunion ¹³	Piton des Neiges Olivine basalt	Piton de la Fouanaise Olivine basalt	30
Tahiti ¹⁴	Tahiti-Nui Ankaramite- basanite	Tairapu Basanite- ankaramite	30
Grande Comore ¹⁴	Kartala Alkali basalt	La Grille Basanite	30
St Helena ²¹	North-east volcano Alkali basalt	South-west volcano Alkali basalt	~10
Prince Edward Islands ¹⁵	Marion Island Alkali basalt	Prince Edward Island Alkali basalt	30
Ba and K enriched relative to PEI alkali basalts			

An important point about the magmas of these volcanic couples is that some of the centres show fundamental petrological differences which cannot arise by low pressure petrological processes. These differences are mainly related to degree of silica saturation or enrichment in large cation trace elements,

for example, Kilauea and Mauna Loa¹², Marion and Prince Edward Islands¹³, alkali basalt-basanite (Grande Comore)¹⁴, differences which are now generally interpreted as arising from high pressure processes such as eclogite¹⁵ or orthopyroxene¹⁶ fractionation or degree of partial melting¹⁷. This point is emphasised by the following discussion of two well documented examples:

The island of Grande Comore lies at the north-western end of a volcanic chain showing classic geomorphological features of age migration, with the oldest in the south-east and youngest in the north-west¹⁸. (I note that if these volcanoes result from plate movement over a hot spot, this motion was from north-west to south-east, about ninety degrees to the motion suggested by Morgan¹ but more or less parallel to that of Burke *et al.*⁵.)

Grande Comore is formed by the coalescence of two volcanoes, Kartala in the south-east and La Grille about 30 km to the north-west. Kartala consists of an alkali basalt suite which shows coherent chemical variations caused by low pressure fractionation of olivine, augite and plagioclase. La Grille consists of a coherent suite of basanites, rich in high pressure ultramafic inclusions, which shows little or no effects of low pressure fractionation. There is a sharp field division between the two volcanoes, and little overlap in chemistry, suggesting independent origins with no mixing of the magmas during ascent. The magmas were interpreted to have arisen from different degrees of partial melting at depths below 60 km, with rapid rise to the surface along conduits definitely less than 15 km in diameter (halfway between the two craters) and probably much smaller¹⁴.

The island of Hawaii consists of five separate volcanic centres separated from each other by distances around 30 km. Like Grande Comore it lies at the youngest end of a volcanic chain which serves as a kingpin in Morgan's mantle plume hypothesis¹. The following discussion is concerned with only Kilauea and Mauna Loa, although in principle it applies to Mauna Kea, Hualalai and Kohala as well.

Both Kilauea and Mauna Loa have tholeiitic magmas, although the latter are depleted in K₂O and other large cation elements¹². Wright¹² recognised two kinds of chemical variation in Kilauea lavas, short term variations between each summit eruption caused by high pressure fractionation of hypersthene from magma produced at depths below 60 km and long term variation resulting from a multistage process of fractionation of some combination of olivine, Cr-spinel, orthopyroxene and clinopyroxene, likewise at high pressures. He further suggests that differences between the 1969 Kilauea lavas and Mauna Loa lavas could be explained by the same high pressure fractionation processes which cause differences within Kilauea lavas. Although I accept Wright's interpretation as a reasonable possibility, it should be pointed out that precisely similar differences would result from differing degrees of partial melting.

Irrespective of the details of high pressure magmatic processes responsible, be they partial melting or crystal fractionation, many of the volcanic edifices above hot spots appear to be formed from two (or more) volcanic centres with fundamental differences in magma chemistry which result from high pressure processes. These magma batches may mix, as for Kilauea and Mauna Loa¹², or Tahiti-Nui and Tairapu¹⁹, or they may separate as in Grande Comore¹⁴. They generally originate at depths below 60 km and ultramafic inclusions in many indicate rapid ascent to the surface through narrow conduits. If Press's model²² of oceanic upper mantle is valid and the low velocity zone is at an average depth of 150 km, and if Morgan¹ is correct in his suggestion that the lithosphere is 'de-coupled' at the low velocity zone, then it must be assumed that individual volcanoes cannot be directly related to mantle plumes as a source of magma, except in the special case where the volcano remained stationary above the plume, as suggested by Schilling⁷ for Iceland. If the plume itself were contributing material to the volcanoes one would not expect such subtle differences to

persist in such precise spatial relationships. Since Iceland-type magmatic differences are found in areas which were not stationary and therefore not fed directly by the mantle plume, one must agree with O'Hara⁸ that a direct mantle plume source is unnecessary to explain the Iceland magmas. Further, these relationships suggest that the magmas did not rise directly from the low velocity zone which would presumably be a zone of homogenisation.

Given that deep mantle plumes are not necessarily direct sources of magma, there is less reason to assume that plumes

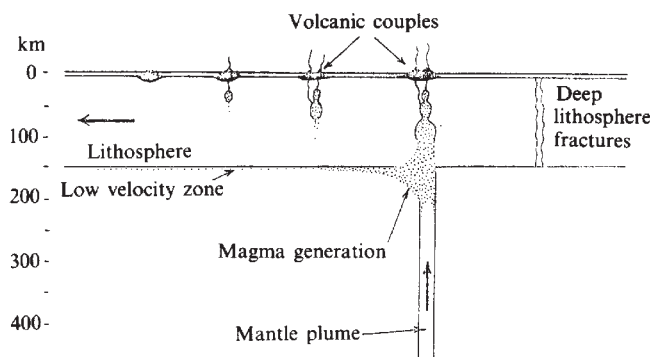


Fig. 1 Magma generated above the mantle plume (either thermal or convective) in or below the low velocity zone is collected in magma chambers and migrates towards the surface after fractionation at a number of intermediate staging areas. Magmas tapped along deep fractures from different staging areas form adjacent volcanoes of different compositions. With migration (to the left) of lithosphere plate above the plume, the magma source is smeared out, the supply of magma is cut off and volcanism is restricted to progressively more extreme fractionation products and eventually terminated entirely, and a new volcanic edifice is formed as other lithosphere 'fractures' (or at least weaknesses) pass over the plume. (Modified from Wilson² and Wright¹².)

are the result of upward mass transport, that is, of mantle convection. The only necessity for the observed linear patterns of magma generation is a concentrated heat source at the base of the lithosphere—a 'hot spot' or 'thermal plume'. These distinctions between mantle plumes and magma generation may satisfy Tozer's request¹¹ for terminological clarification, although it is clearly up to the geophysicists to answer some of the resulting questions. For example, do the gravity anomalies which Morgan¹ explained as resulting from mantle plumes have any equally reasonable alternative explanations? Is it possible to have thermal plumes without mass transport, perhaps by zones of high thermal conductivity or concentrated diffusion?

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Lyoluminescent tissue equivalent radiation dosimeter

WHEN some irradiated solids are dissolved in water or certain other solvents, a light emission takes place which, following Wiedemann¹, we call "lyoluminescence." With inorganic materials such as alkali halides, the reactions of trapped electrons from F centres are responsible for the light emission². In the case of some organic materials such as saccharides, trapped free radicals are involved in the process of lyoluminescence^{3,4}.

It is possible to measure doses between 1 and 10^7 rad with an accuracy of 5% using NaCl and water as a solvent⁵. The stored lyoluminescent energy in NaCl decreases only by 15% after 7 month of storage but it is sensitive to optical and thermal bleaching. Furthermore, the effective atomic number of NaCl is approximately 16, differing considerably from that of human tissue (about 7.5).

The investigation of monosaccharides including glucose, xylose and mannose has demonstrated the stability of the trapped free radicals. There was no decrease in their lyoluminescence over 7 month. Their performance has been evaluated with respect to the sensitivity to radiation and stability against thermal annealing. They show linear dependence with doses up to 100 krad. The lower dose detected by the apparatus used (Fig. 1) was 100 rad (ref. 6).

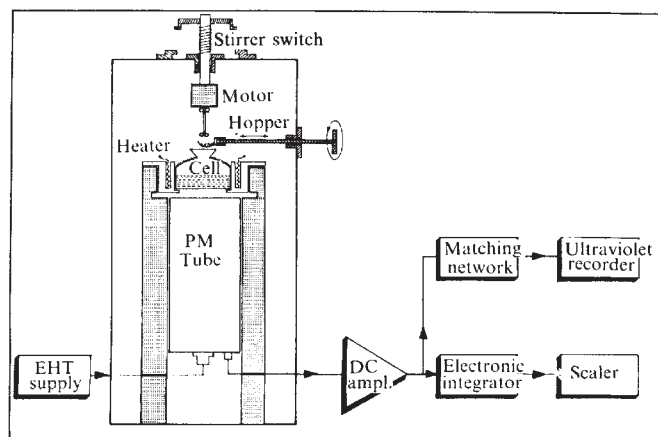


FIG. 1 The lyoluminescence reader

From the shape of the emitted light decay curve, it is evident that there are two or more diffusion controlled reactions involved which differ in their time scale. Here we

deal with the short lived component (integrated for 3 s) because it has a much greater intensity than the long lived one.

With NaCl, when using TiCl or fluorescein solution instead of water, the enhancement of light takes place but only at higher doses. With saccharides these fluorescent systems have no effect.

It would be possible to use the lyoluminescence of saccharades for clinical dosimetry if their sensitivity could be enhanced. Therefore the chemiluminescent reactions of luminol (3-aminophthalhydrazide) with oxidising agents was utilised^{7,8}. Using a ^{60}Co - γ -source, irradiated saccharides give a bright blue light ($\approx 4,460 \text{ \AA}$) when they are dissolved in luminol solution. The light enhancement compared to that of water is about 10^6 times. The best results were obtained with trehalose. $2\text{H}_2\text{O}$ (Fig. 2). It shows an approximately linear light output between 1 and 60^6 rad, and the lower limit is probably controlled by the level of impurities in the system, as luminol is sensitive to metallic ions such as Cu^{2+} and Co^{2+} in higher oxidation states. The slope of the curve is dependent upon the concentration of luminol and the pH of the solution.

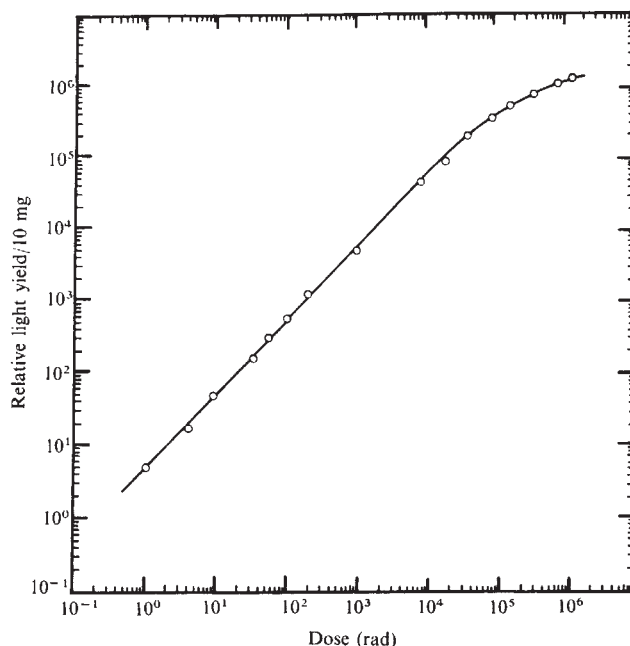


FIG. 2 Integrated light output versus radiation dose, when trehalose. $2\text{H}_2\text{O}$ was dissolved in 5 ml of luminol solution (125 mg luminol, 1.25 g Na_2CO_3 and 2.5 mg chlorohemine in 1 l deionised water).

It is quite likely that the oxidising species responsible for exciting luminol are formed as a result of free radical reactions with dissolved or adsorbed oxygen in the system. For example, (ROO) is capable of oxidising luminol which is accompanied by the emission of light. Oxidising species were, however, reported to be formed when irradiated saccharides are dissolved in water, and the possibility of H_2O_2 or ROOR formation is not ruled out^{9,10}. Thus trehalose. $2\text{H}_2\text{O}$ which is a fairly true tissue equivalent material makes a good candidate for lyoluminescence dosimetry.

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Newfoundland Ophiolites and the Geology of the Oceanic Layer

THE significance and origin of ophiolites is a matter of interest to geologists. Many investigators interpret ophiolites as slices of oceanic crust which have been tectonically emplaced in orogenic belts¹⁻⁷. Moores and Vine⁵, Dewey and Bird⁶, and Coleman⁷ have proposed just such an origin for ophiolites as geographically diverse as Cyprus, Newfoundland and the Western United States. If ophiolites are representative of the geology of the oceanic crust, then they provide direct access to samples of oceanic crust and the underlying upper mantle—rocks at present inaccessible except for the fortuitous catch in dredge hauls located on tectonic escarpments of the ocean floor. Although we accepted the proposition that ophiolites, if well preserved and not altered by events unrelated to lithosphere accretion, are representative of oceanic crust, we wished first to test this proposition by observing whether representative ophiolite samples had measured velocities compatible with the seismic layering defined for oceanic crust. If the velocities of ophiolite samples were compatible with the range of seismic velocities recorded for oceanic crust, then we hoped to use the ophiolite measurements to understand better the details of the seismic layering of oceanic crust. Here we summarise the acoustic velocity data obtained from metadolerites, gabbros and cumulate gabbros from the Mings Bight ophiolite

assemblage⁶ located along the northern coast of Newfoundland. A more detailed discussion of these ophiolite samples (J. J. P., in preparation) will give a thorough presentation of petrography, sample density and measured compressional wave velocity of each sample at pressure intervals from 1 atm to 7 kbar.

Seismic models of the oceanic crust have been formulated principally from the interpretation of seismic reflection and refraction data. The velocity structure of the oceanic crust consists of three layers⁸. The oceanic layer (layer 3) is characterised by velocities in the range 6.7 to 6.9 km s⁻¹. Although most reported velocities for the oceanic layer are in this range, the full range of reported velocities is 6.1 to 7.0 km s⁻¹. Overlying this is oceanic basement (layer 2), which exhibits a broad range of velocities (3.5 to 6.0 km s⁻¹), most of which lie in the range 4.4 to 5.6 km s⁻¹. The uppermost layer (layer 1) consists mainly of consolidated and/or unconsolidated sediments having velocities of 1.5 to 3.5 km s⁻¹. Recent seismic refraction studies, however, indicate that the velocity structure of the oceanic crust is more complex than the early studies suggested. Sonobuoy refraction studies of the Reykjanes Ridge show that oceanic basement (layer 2) is made up of two velocity layers⁹. The upper portion (layer 2A) has a recorded velocity range of 2.33 to 3.84 km s⁻¹. The lower portion (layer 2B) is characterised by a velocity range of 4.05 to 5.92 km s⁻¹. Sonobuoy refraction studies in the Pacific show that the oceanic crust (layer 3) is made up of two velocity layers^{10,11}. The upper layer is 2 to 3 km thick and has a velocity range of 6.5 to 6.8 km s⁻¹ and overlies a lower layer 2 to 5 km thick with a recorded velocity range of 7.0 to 7.7 km s⁻¹ (average 7.4 km s⁻¹).

To interpret these seismic models of oceanic crust in terms of lithological units, it is necessary to measure in the laboratory the physical properties of representative samples of rock types recovered from oceanic escarpments. Comparisons of seismic refraction results with laboratory measurements at confining pressures equivalent to *in situ* lithostatic pressures provide information about the possible composition of velocity layers. Several investigators have made laboratory measurements on oceanic samples and have proposed geological models of the oceanic crust. Based on studies of basalts, meta-basalts and meta-gabbros, Christensen¹², Christensen and Shaw¹³ and Barret and Aumento¹⁴ proposed that the oceanic crust is composed of basalt and meta-basalt (layer 2) which overlies meta-gabbro (layer 3). We have recently presented the results of a study of basalts, meta-basalts, meta-gabbros, gabbros and serpentinites recovered from the escarpments of the Kane

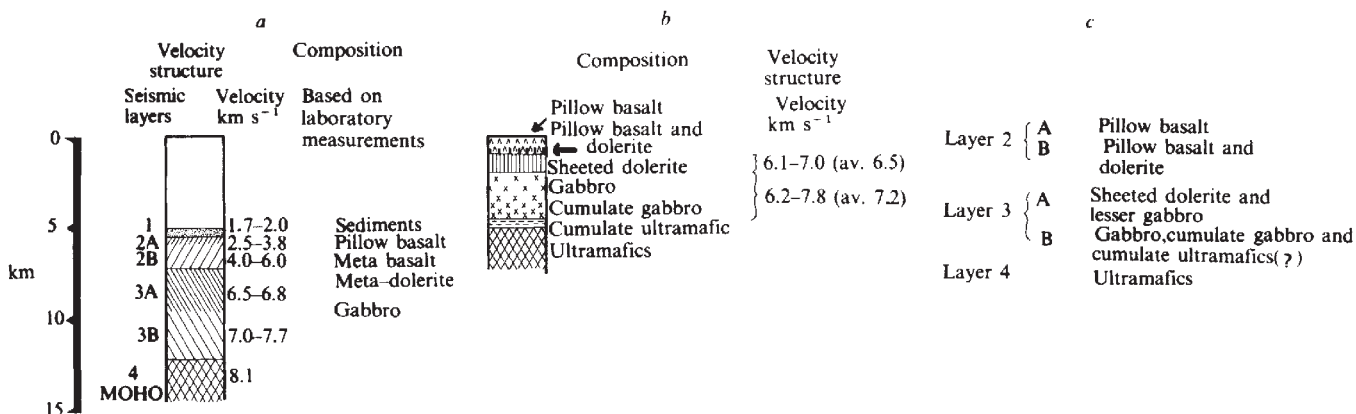


Fig. 1 *a*, Generalised velocity structure of the oceanic crust and the inferred composition of the oceanic crust based on a correlation of velocities measured in the laboratory on dredged rocks with velocities measured by seismic refraction methods. *b*, Generalised geology of Mings Bight ophiolite complex and the measured velocity range of representative dolerite and gabbroic samples. *c*, Correlation between Mings Bight ophiolite assemblage and the layering of the oceanic crust based on laboratory compressional wave velocity measurements.

Fracture Zone¹⁵. Our results, when integrated with the standard velocity structure of the oceanic crust, led us to propose a slightly different model for the oceanic crust (Fig. 1a). We proposed the following structure from top to bottom: layer 2A (2.5 to 3.8 km s⁻¹ is made up of a fractured pile of glassy pillow basalts; layer 2B (4.0 to 6.0 km s⁻¹) is made up of zeolite and greenschist facies meta-basalt and minor meta-dolerite; layer 3 (6.7 to 6.9 km s⁻¹) is made up predominantly of unaltered gabbro. The measured velocities of meta-gabbro (greenschist and amphibolite facies), serpentinised peridotite and actinolite-rich rocks were not compatible with the generalised velocity structure of the ocean basins, suggesting that these rock types do not occur in abundance within the oceanic crust but instead reflect tectonic processes associated with the escarpment on which they were sampled. We were intrigued with the seismic refraction results of Maynard¹⁰ and Sutton *et al.*¹¹ which indicated a high velocity layer (>7.0 km s⁻¹) in the lower portion of layer 3. Although only one of the measured gabbro samples had a velocity of more than 7.0 km s⁻¹, we suggested that cumulate gabbros or pyroxenites could be responsible for the high velocity component of layer 3. Rock types of this character had been reported from some fracture zones^{16,17}.

The ophiolite samples we studied were obtained from the Mings Bight ophiolite complex in north-eastern Newfoundland. These rocks form a steeply dipping zone between the Fleur de Lys metamorphic complex of Cambrian age and Lower Devonian clastics and volcanic rocks. The ophiolite rocks show tectonic contacts and appear to have been tectonically emplaced during the Middle Devonian¹⁸. This location is ideal because it represents a fully developed ophiolite complex in which all units (see Fig. 1b) are represented and in which the rocks have been relatively unaffected by metamorphic episodes associated with the emplacement of the assemblage. Also, as a consequence of locale and climate, ice wedging and wave action cause the altered rock surface to be spalled off and washed away exposing unweathered rock for sampling.

Depending on the sample size, two or three cores, whose axes were oriented so that they were mutually perpendicular, were cut from each sample using a small (0.5 inch) diamond corer. Specimen lengths ranged between 1 and 2.5 inch. The ends of each core were cut smooth and parallel to within 0.001 inch of each other, yielding right circular cylinders. The specimens were jacketed in thin copper foil (2 ml thick) and all joints were sealed with soft solder. The main function of the copper jacket was to prevent entry of pressurising fluid into the pores and cracks of the specimen, so that measurements were made under zero pore pressure, and to serve as electrical shielding, thereby improving the signal-to-noise ratio. To measure the travel time of acoustic energy transmitted through the specimens, a variation of the pulse transmission method of Birch¹⁹ as described by Mottaboni and Schreiber²⁰ was used. A precision of 1% could be obtained.

The velocity of sound propagated through rocks at low confining pressures depends on the presence of pores and cracks and on their geometry^{21,22} resulting in the measurement of markedly lower velocities than would be measured for theoretically dense materials of the same composition. For this reason velocity was measured as a function of hydrostatic pressure. Typically, the greatest effect on velocity occurs within the first 2 kbar of applied pressure. The slope of the velocity curve asymptotically approaches that for theoretically dense material at confining pressures in excess of 2 kbar. The velocities reported here are those determined at 1.5 kbar, which is within the range of *in situ* lithostatic pressure for the oceanic layer (layer 3A, 3B). The density of the specimens was calculated from direct measurements of their lengths, diameters and masses, and is regarded as accurate to within 1%.

The results of thirty-two measurements on thirteen samples of gabbro and cumulate gabbro are shown in Fig. 2. We

display the individual measurements, rather than averaged results, to emphasise the spread in the velocity range that might be measured for a given rock type. The leucocratic to melanocratic gabbros commonly exhibit ophitic textures and are composed in general of calcic plagioclase and clinopyroxene with minor vein and interstitial serpentine, fibrous amphibole and chlorite. The plagioclase of the gabbros is often saussuritized. The cumulate gabbros are characterised by bands enriched in pyroxene and olivine within the gabbro. Minor serpentine and fibrous amphibole are associated with the cumulate gabbros. The calculated densities of the gabbro and cumulate gabbro specimens range from 2.9 to 3.2 g cm⁻³. The compressional wave velocity range of these gabbroic samples at 1.5 kbar confining pressure is 6.1 to 7.8 km s⁻¹. The majority of the samples have measured velocities above 6.9 km s⁻¹ with a well defined peak at 7.4 to 7.5 km s⁻¹. The six samples with velocities below 6.9 km s⁻¹ exhibit a larger amount of fibrous amphibole and chlorite than the higher velocity samples. We observed in a study of oceanic gabbro

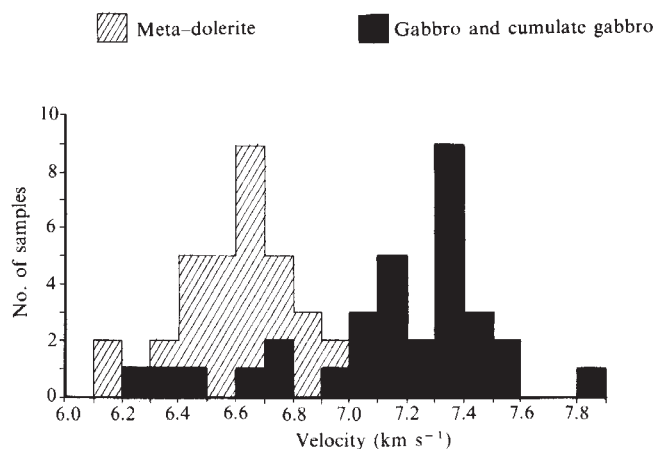


Fig. 2 Histogram of measured velocities for thirteen samples of gabbro and eleven samples of meta-dolerite (greenschist facies) from Mings Bight ophiolite complex. Two or three specimens, oriented so that they were mutually perpendicular, were cut from each sample. The measured velocity for a given specimen displayed individually. The velocity results shown here were measured at a confining pressure of 1.5 kbar which is within the range of *in situ* lithostatic pressure for the oceanic layer (layer 3).

that the development of amphibole and chlorite at the expense of primary mineral phases decreases the compressional wave velocity¹⁵.

The results of twenty-seven measurements on eleven samples of greenschist facies meta-dolerite are shown in Fig. 2. The meta-dolerites often exhibit relict sub-ophitic textures and are composed of plagioclase, sometimes enriched in Na, and pyroxene with varying amounts of epidote, chlorite, actinolite and quartz. The calculated densities of the meta-dolerite samples vary from 2.8 to 3.0 g cm⁻³. The compressional wave velocity range of the dolerites at 1.5 kbar confining pressure is 6.1 to 6.9 km s⁻¹. At a confining pressure of 0.75 kbar (calculated *in situ* lithostatic pressure range for layer 2) the velocities of the dolerites range from 6.0 km s⁻¹ to 6.9 km s⁻¹, indicating that dolerites of the sheeted dike layer are not representative of seismic layer 2B (velocity range of 4.0 to 6.0 km s⁻¹).

The measured compressional wave velocities of the dolerite and gabbro samples range from 6.1 to 7.8 km s⁻¹. Seismic refraction measurements of layer 3 (A and B) of the oceanic

crust show a similar range of velocities⁸. This correlation supports the interpretation that ophiolite assemblages are representative of oceanic crust. The histogram of measured compressional wave velocities (Fig. 2) exhibits a distinct bimodality with a dolerite peak at 6.6 to 6.7 km s⁻¹ and a gabbroic peak at 7.4 to 7.5 km s⁻¹. These velocities compare well with the bimodal distribution of seismic velocities recorded for layer 3A (6.5 to 6.8 km s⁻¹) and 3B (7.0 to 7.8 km s⁻¹)^{10,11}. Accepting the proposition that ophiolites are representative of oceanic crust, we conclude from our data that the upper portion of the oceanic layer (seismic layer 3A) is composed predominantly of sheeted dolerite and possibly some gabbro. The lower portion of the oceanic layer (seismic layer 3B) is composed predominantly of gabbro and cumulate gabbro. Based on velocity alone it is difficult to say conclusively whether the cumulate ultramafic rocks (pyroxenites) belong with layer 3B or upper mantle. We sampled several pyroxenites for this study but all the samples have been serpentinised, resulting in anomalously low velocity. Some published values of unaltered pyroxenites at 1.5 kbar confining pressure indicate that rocks of this type have velocities in the range 7.3 to 7.7 km s⁻¹ and therefore could be a constituent of layer 3B. More work is needed to define the velocity range of pyroxenites but, based on the results to date, we propose that the cumulate ultramafics belong with the gabbroic rocks of layer 3B.

Poster²³ has recently reported results on velocity measurements of the Troodos ophiolites. His results for three gabbros are similar to those reported here; two of the three diabase measurements, however, are lower than those reported here, presumably because of greater alteration. Nonetheless, he arrived at a conclusion similar to our model. If the ophiolite assemblages are analogues of the geology of the oceanic crust, then it is clear that the petrological associations within the oceanic basement and oceanic layer are complex. Indirect geophysical measurements of the physical expression of the oceanic layers do not have the resolution to resolve the inherent complexity of these layers and therefore careful study of ophiolite assemblages may hold the key to the details of oceanic lithosphere geology.

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Molecular Complexity of Water Vapour and the Speed of Sound

SEVERAL real atmosphere and laboratory spectroscopic measurements¹⁻³ suggest that water vapour near saturation contains polymeric molecules of which the concentration rises with increasing pressure and decreasing temperature. Existing PVT data which could support this conclusion lack the necessary accuracy for temperatures which are approaching atmospheric values and no related speed of sound measurements seem to be available for the conditions of interest.

We give here a preliminary report of some new measurements derived from observations of the transit time of sound pulses in water vapour. The measurements were made at a fixed temperature and the vapour pressure was varied from low values up to values close to saturation. Results are shown in Fig. 1.

A gas such as neon, which shows no molecular association, gives speed values which are independent of pressure, so the trend we observe in water vapour of speed decreasing

with increasing pressure is taken as showing that it contains molecular complexes. The speed decrement with pressure becomes greater as temperature is lowered. To compare with the observations we have considered a model in which the complexes are all taken to be dimers. This model will be represented by straight lines in the plot since the speed of sound varies as the square root of gas density and dimer concentration will vary as its square. The fractional dimer concentration is measured by the slope of these lines and at 50° C and 90% saturation we find a value of 8×10^{-3} . The temperature dependence of the slope is a measure of the dimer binding energy for which a value of 0.5 ± 0.1 eV per dimer molecule is found.

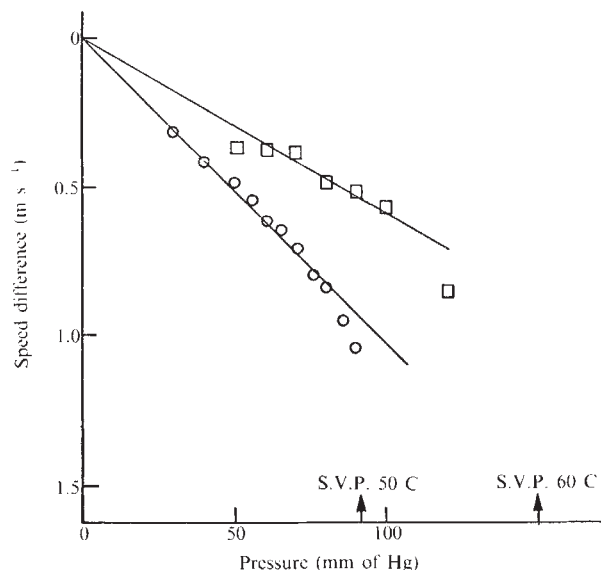


Fig. 1 Speed of sound in water vapour. The ordinate is the difference between the observed speed and the speed, appropriate to the temperature, extrapolated to zero pressure. □, 60° C; ○, 50° C.

The model used for analysis is only a first approximation and is probably oversimplified: the values also are tentative and subject to confirmation when the measurement technique can be refined, but these speed of sound measurements clearly show molecular complexity in water vapour at temperatures approaching tropospheric values.

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Light Sensitivity of Tests for Cadmium on Ceramic Tableware

MANY countries, including Ireland, Great Britain and the United States, have introduced national standards aimed at controlling the amount of lead and cadmium which can be leached into food from ceramic tableware¹⁻³.

Most countries including those mentioned above favour a cold test while others, notably Denmark, favour a boiling test of shorter duration⁴. The cold test usually involves filling the article with 4% acetic acid and allowing it to stand for 24 h. Limits are set for the concentration of lead and cadmium in the resultant leaching solution. In the Irish and United States tests, the limits are set at 7 p.p.m. for lead and 0.5 p.p.m. for cadmium. The British Standard (BS) for ceramic flatware is 20 and 2 p.p.m. for lead and cadmium respectively, those for hollow ware below 1,100 ml are 7 and 0.7 p.p.m. for lead and cadmium respectively and the BS limits for hollow ware of 1,100 ml and above are 2 and 0.2 p.p.m. for lead and cadmium respectively. The method of test is, however, very similar to that used in the American and Irish tests.

The series of experiments reported here were carried out on whiteware pottery with a lead-bearing glaze on which was applied a multicoloured silk-screen onglaze pattern, known to contain cadmium. A series of plates, decorated, heat treated and tested at room temperature in a similar manner in the absence of light, gave values of 0.17 ± 0.06 p.p.m. for cadmium. But, if plates from a similar batch were tested under daylight conditions or under artificial light at similar temperatures, results up to 2.5 p.p.m. could be recorded depending on the duration, intensity and wavelength of the radiation allowed to fall on the plates in the 24 h period. The results also indicate that significant leaching can occur in brightly lit conditions when the 4% acetic acid solution is replaced by deionised water as the leaching solution. Results of 0.5 p.p.m. have been recorded, once again depending on the duration and intensity of the light in the 24 h period.

Throughout the experiments no change was noted in the results for the amount of lead leached, which remained at 1.2 ± 0.4 p.p.m. When distilled water was used, no lead was leached into solution.

There is also evidence which indicates that the boiling test used in Denmark can be similarly light sensitive.

We suggest that, in the case of the series of results reported here, the light sensitivity of the test is due to the photo-oxidation of insoluble cadmium sulphide to soluble cadmium sulphate. Cadmium sulphide has been shown by X-ray diffraction to be present in the onglaze decoration. Clearly, articles may pass or fail the various national specifications according to the light conditions in which the test is carried out. Further details of the results quoted here will be published elsewhere.

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BIOLOGICAL SCIENCES

Absorption of Cadmium, Copper and Zinc by Dogwhelks in the Bristol Channel

SEVERAL independent studies have established that unusually high concentrations of certain heavy metals, most notably cadmium, are found in littoral flora and fauna of the Bristol Channel¹⁻⁴. An experiment by Peden *et al.*⁵ established that the concentrations of cadmium and zinc in dogwhelks (*Nucella lapillus*) did not markedly decrease after transferring specimens to clean water for seven weeks. Bristol Channel dogwhelks have shells with an elongated shape, clearly distinguishable from the usual shell shape. This feature, together with the fact that dogwhelks move very little (J. H. Crothers, personal communication), makes the species ideal for studying the absorption of metals.

In April 1973, 120 dogwhelks with the usual shell shape

Specimens were collected after 1 week and thereafter at monthly intervals. The survival rate was good, although some fatalities were noticed at the end of June, when the spring tides uncovered the animals in early afternoons in hot sunlight. A similar proportion of fatalities was noticed among the local dogwhelks. Even after 6 months no specimen was found outside the release area.

After removing the shell, each specimen was weighed, dried at 105°C for 24 h, reweighed and dissolved in 5 cm³ concentrated nitric acid (AR grade). The solution was diluted to 25 cm³ with double-distilled water, and the metal concentrations were determined by atomic absorption spectrophotometry (Table 1).

Several common limpets (*Patella* sp.) were collected from Beer at the same time as the dogwhelks. The shells were scratched with a cross and released at St Andrew's Head on a rock in the *Fucus serratus* zone. Local limpets from the same spot were collected at the time of transplanting the specimens. The survival rate was poor and the small collections were made at irregular intervals (Table 2).

The concentration of cadmium in the dogwhelks increased at an appreciably slower rate than that of zinc. At the end

Table 1 Concentrations of Cd, Cu and Zn in *Nucella lapillus* Transferred from Beer (Dorset) to St Andrew's Head (North Somerset)

Sample details	Date	No. of specimens	Cd	Cu	Zn
Beer, before transplant	April 8, 1973	10	36 (11-62)	70 (20-110)	345 (110-480)
	Standard deviation		14	23	95
Transplanted specimens	April 18, 1973	6	40 (19-74)	140 (60-260)	620 (190-1,150)
	Standard deviation		17	74	400
Transplanted specimens	May 6, 1973	10	63 (44-93)	175 (70-370)	1,400 (890-2,350)
	Standard deviation		18	82	450
Transplanted specimens	June 2, 1973	6	92 (62-140)	120 (80-180)	2,000 (1,360-6,450)
	Standard deviation		27	33	2,000
Transplanted specimens	July 1, 1973	6	135 (117-150)	113 (78-150)	2,800 (1,700-3,600)
	Standard deviation		12	29	600
Transplanted specimens	July 29, 1973	7	184 (132-226)	200 (105-330)	3,200 (1,800-4,400)
	Standard deviation		34	78	850
Transplanted specimens	August 27, 1973	6	211 (167-243)	170 (80-390)	2,530 (1,700-4,330)
	Standard deviation		27	100	950
Local specimens	December 1972	10	460 (270-580)	740 (405-1,010)	3,090 (1,300-4,500)
	Standard deviation		105	180	1,100
Local specimens	August 28, 1973	11	780 (500-1,120)	905 (385-1,750)	2,900 (1,460-4,030)
	Standard deviation		200	380	1,050

Mean values (with extreme values in parentheses), p.p.m. in the dry flesh.

Table 2 Concentrations of Cd, Cu and Zn in *Patella* sp. Transferred from Beer (Dorset) to St Andrew's Head (North Somerset)

Sample details	Date	No. of specimens	Cd	Cu	Zn
Beer, before transplant	April 8, 1973	11	11 (3.5-28)	7 (3.5-12)	91 (55-130)
Transplanted specimens	April 15, 1973	4	10 (3.5-17)	11 (8-18)	115 (90-140)
Transplanted specimens	May 6, 1973	3	14 (10-20)	17 (15-22)	185 (170-205)
Transplanted specimens	July 29, 1973	2	200 (100, 300)	30 (28, 33)	235 (235, 235)
Local specimens	December 1972	9	220 (67-440)	30 (ND-50)	340 (290-41)

Mean concentrations (with extreme values in parentheses), p.p.m. in dry flesh. ND, Not detected.

were collected at Beer, in Dorset (National Grid reference SY235895). Ten specimens were analysed for cadmium, copper and zinc, and the rest were released in a clearly identifiable area of approximately 500 m² at St Andrew's Head, North Somerset (National Grid reference ST324662). The area was below the *Fucus serratus* zone (below the Mean Low Water Level), where the local dogwhelks live, feeding on acorn barnacles (*Elminius modestus* is the dominant species here).

of the fourth month, the cadmium concentration was significantly lower than that in the native specimens, but it was still increasing steadily. By the end of the second month, the zinc concentrations were statistically indistinguishable from those in the native specimens. The copper concentrations remained considerably less than those in the native specimens.

Because of the small number of specimens analysed, only speculative observations about the uptake of metals by limpets

can be made. But it does seem that limpets absorb both copper and cadmium more rapidly than dog whelks.

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Use of Sulphur-coated Urea for Revegetation of Blast Furnace Slag

APART from studies in the Lower Swansea Valley¹⁻⁴ there has been no attempt to investigate the factors inhibiting plant growth on blast furnace slag. This material is probably second only to colliery spoil as a mineral waste problem in the United Kingdom.

Recent investigations on the revegetation of blast furnace slag in Lancashire have shown that growth is limited by high pH (10.2 to 10.5) and serious deficiencies of the major nutrients nitrogen and phosphate (my unpublished work). Although exposure of the waste causes a reduction in pH at the surface due to leaching of hydroxides, the underlying material remains highly alkaline (Table 1). Consequently, plant growth is restricted by the limited depth of the hydroxide-free rooting substratum. The waste is also highly porous so that soluble nutrients such as nitrogen are quickly lost from the planting medium.

The provision of adequate phosphate for growth establishment is not difficult and some slags may even contain this nutrient. For the reasons described, however, soluble inorganic forms of nitrogen are most inefficient when used on this waste. I have therefore investigated the efficiency of sulphur-coated urea as a slow release nitrogen source in order to achieve a visually acceptable grass cover on the waste without involving the provision of expensive manurial or soil top coverings.

A randomised block experiment was set up on a levelled tip site with a surface pH of 9.0 after some 4 months of exposure. Five different nitrogen treatments were investigated, namely, nil N, 50 and 100 kg ha⁻¹ N as urea, and 50 and 100 kg ha⁻¹ N as sulphur-coated urea. Phosphate was applied to all the plots at 75 kg ha⁻¹ P₂O₅ and *Festuca rubra* which is relatively

kg ha⁻¹ N as sulphur-coated urea. In this way, the efficiency of slow-release nitrogen was investigated as an initial and maintenance fertiliser treatment.

All the plots were assessed visually by four independent observers in late May 1973. The criterion of assessment was overall visual acceptability as affected by percentage cover of the substratum, colour and uniformity of growth, and sward density. Each of the experimental grass plots was designated an index of acceptability within the range 1 to 10 and the results were assessed by statistical analysis.

Table 2 shows the mean indices of growth acceptability in relation to the initial and secondary nitrogen treatments. It can be seen that overall growth was still influenced by the initial nitrogen applications at this time. The residual slow-release nitrogen responses were significantly better than

Table 2 Mean Indices of Visual Acceptability as Affected by Nitrogen Fertilisation

Initial N treatments	Nil	N1	N2	SN1	SN2
	3.30	3.83	4.43	4.73	5.37
	l.s.d. at $P=0.05=0.53$				
Second N treatments	Nil	N		SN	
	1.54	4.94		6.52	
	l.s.d. at $P=0.05=0.41$				

N, Urea nitrogen.

SN, Sulphur-coated urea nitrogen.

those obtained by the use of soluble urea nitrogen. It is interesting that the performance from the low rate of slow-release nitrogen was superior to that from the high rate of urea nitrogen. Considering the effects of the second fertiliser treatments, slow-release nitrogen was again significantly better than uncoated urea. Therefore, both initial and maintenance applications of slow-release nitrogen give better growth than soluble nitrogen.

Table 3 shows the interactions of the initial and second nitrogen treatments. Clearly, nitrogen is better applied after planting if one fertiliser treatment only is involved. For good growth, however, it is obviously necessary to provide two additions, both of which should be in slow-release form for best results. If soluble and slow-release nitrogen treatments are combined, it is preferable to use the slow-release form at the second fertilisation rather than the first.

Table 3 Mean Indices of Visual Acceptability in Relation to Initial and Maintenance Nitrogen Regimes

		Maintenance N		
		Nil	N	SN
Initial N	Nil	1.00	3.80	5.10
	N	1.00	4.80	6.60
	SN	2.35	5.65	7.15
		l.s.d. at $P=0.05=0.91$		

Indices for initial nitrogen treatments are means of high and low levels of application.

tolerant of the waste conditions⁴ was sown in June 1972. In early September 1972 there was evidence of phosphate deficiency. Accordingly, the plots were refertilised with 125 kg ha⁻¹ P₂O₅ and three nitrogen treatments were superimposed on the initial treatment regimes according to a split plot design. These were nil N, 100 kg ha⁻¹ N as urea, and 100

The critical limit of visual acceptability at the time of assessment was represented by an index of about 6.0. Since this was only exceeded following treatment with slow-release nitrogen, it is clear that the choice of slow-release against soluble nitrogenous fertiliser can determine the success or failure of acceptable vegetation establishment on blast furnace slag. These results may have important implications in the revegetation of

Table 1 pH Profile of Blast Furnace Slag after 12 Months Exposure

Depth (cm)	pH
0	8.1
2.5	9.0
5.0	10.2
7.5	10.3
10.0	10.5
15.0	10.4

other industrial waste materials where similar problems of substrate toxicity and adverse nutrient retention are involved.

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N-terminal Acetylation of Histone-like Nascent Peptides on Rat Liver Polyribosomes *in vitro*

SEVERAL proteins are known to have the amino terminus blocked by acetyl radicals¹. At least six of these proteins, including the f_{2a} histones, have an N-acetylserine terminus². The mechanism by which the acetyl group becomes attached to these molecules is not well understood. Proposals suggesting the initiation of protein synthesis by acetylated amino acids as a general mechanism in eukaryotes³ have been refuted⁴. However, a role of N-acetyl amino acids in the initiation of the synthesis of certain specific proteins cannot be ruled out⁵. An explanation for the presence of N-acetyl amino acids at the amino terminus of proteins is the existence of an acetylating enzyme specific for certain N-terminal sequences in proteins or growing polypeptides chains. The finding of an acetyltransferase activity bound to rat liver ribosomes and specific for the N-terminal amino acid of some proteins associated with the ribosomes⁶ offers an appropriate system for the study of the alternative mechanism. We report here the analysis of histone-like nascent peptides acetylated *in vitro* in liver polyribosomes prepared from rats hepatectomised 22 h before being killed. The results indicate that acetyl groups can be attached directly to N-terminal serine residues of nascent peptides in the absence of the initiation of protein synthesis.

The proposed role of N-acetylserine tRNA as an initiator of the synthesis of f_{2a} histones results from the finding of N-acetylserine in growing peptide chains isolated from regenerated rat liver and the finding of N-acetylserine tRNA in the cytoplasmic extracts from the same source⁵. The first of these two observations indicates that acetyl groups are attached before histone synthesis is completed, but it does not necessarily imply an initiation mechanism, since the N-terminal acetylation of polypeptide chains during chain elongation gives the same result. To explore this possibility, polyribosomes prepared from rats partially hepatectomised 22 h earlier were acetylated *in vitro* by incubation with 1-¹⁴C-acetyl coenzyme A (ref. 9). Polyribosomes were also acetylated *in vivo* with ³H-sodium acetate⁸ under conditions in which N-terminal acetylation of histone nascent peptides is known to occur⁵. Aliquots of polyribosomes acetylated *in vivo* and *in vitro* were analysed by sodium dodecyl sulphate-acrylamide gel electrophoresis to compare the distribution of acetyl radicals among the proteins resolved in this system. As Fig. 1 shows, two groups of acetyl proteins with molecular weights of 30,000 and 10,000–20,000 were present in polyribosomes acetylated *in vivo* and *in vitro*. We also found that most proteins acetylated *in vivo* had a molecular weight greater than 30,000, and no correspondence with the proteins acetylated *in vitro*. The reason for this disparity is not known.

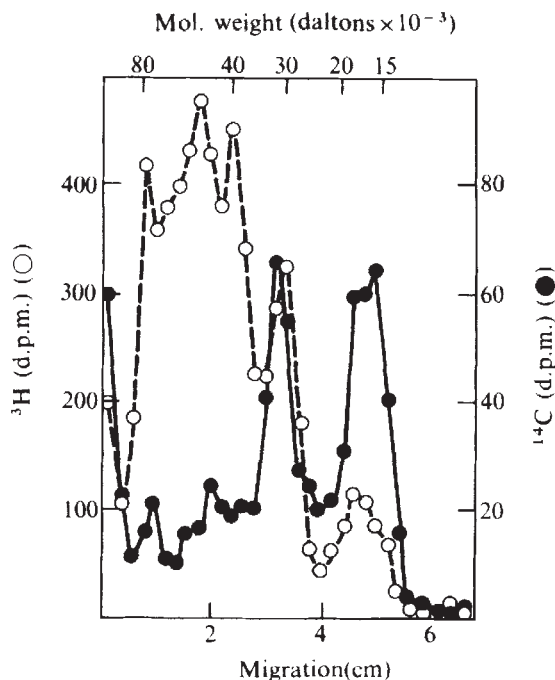


Fig. 1 Sodium dodecyl sulphate acrylamide gel electrophoresis of polyribosomes acetylated *in vivo* and *in vitro*. Liver polyribosomes were prepared⁷ from rats 22 h after partial hepatectomy. One group of rats was given 10 mCi per rat of ³H-sodium acetate before death to obtain polyribosomes acetylated *in vivo*⁵. Polyribosomes from untreated rats (250 A₂₆₀ units) were acetylated *in vitro* by incubation with 0.05 mCi of 1-¹⁴C-acetyl coenzyme A in the presence of 0.05 M Tris-HCl, pH 7.5, 0.1 M KCl and 1 mM dithioerythrol⁹. Aliquots (4–5 A₂₆₀ units) were analysed in 10% acrylamide gels by electrophoresis in the presence of 0.1% sodium dodecyl sulphate and the radioactivity in the gel fractions was counted as described⁹. The solid and open circles correspond to ¹⁴C and ³H-radioactivity, respectively.

To study the presence of acetyl radicals in the histone-like nascent peptides, we incubated the polyribosomes with puromycin in the presence of a high concentration of salt and they were then pelleted by high speed centrifugation. The growing peptides released by puromycin were then treated with 0.25 N sulphuric acid to extract the basic proteins and polypeptides, which were then dialysed for 48 h against 0.025 N sulphuric acid, precipitated with 4 volumes of absolute ethanol in the cold and dried under vacuum. (About 2.5 mg of dried product is recovered from 20 g of wet liver.) In terms of radioactivity, the recovery was less than 5% of the total radioactivity found in polyribosomes. The basic protein and growing polypeptides in the acid extract were characterised in two acrylamide gel electrophoretic systems. In sodium dodecyl sulphate electrophoresis (Fig. 2A), all the acetyl radioactivity was found associated with proteins having apparent molecular weights of 10,000–20,000; in this range of molecular weights is found histone f_{2a} (ref. 11) or its nascent peptides near completion. Electrophoresis in the presence of urea at pH 2.5 (Fig. 2B) revealed that most of the acetyl protein in the sample had a mobility close to the histone standards. From this study we conclude that most of the acetyl protein released from polyribosomes by puromycin and extracted by dilute sulphuric acid represents histone nascent peptides near completion; moreover, the results also indicate that histone nascent peptides on polyribosomes can be acetylated *in vitro*.

The acetyl groups in the histone nascent peptides were characterised by a combination of ion exchange filtration and paper electrophoresis and chromatography. The histone nascent peptides from polyribosomes acetylated *in vitro* and *in vivo* were digested with trypsin, pronase and carboxy-

peptidase⁹. The resulting solution of acetyl amino acids and peptides was passed through a Dowex 50 H⁺ column and the non-absorbed material was eluted with water to obtain the 'acidic' fraction, which contains N-terminal blocked amino acids and peptides. All the absorbed material was eluted with 0.5 N ammonium hydroxide to obtain the 'basic' fraction, containing all the free amino acids and peptides. Radioactivity measurement of these fractions indicates (Table 1)

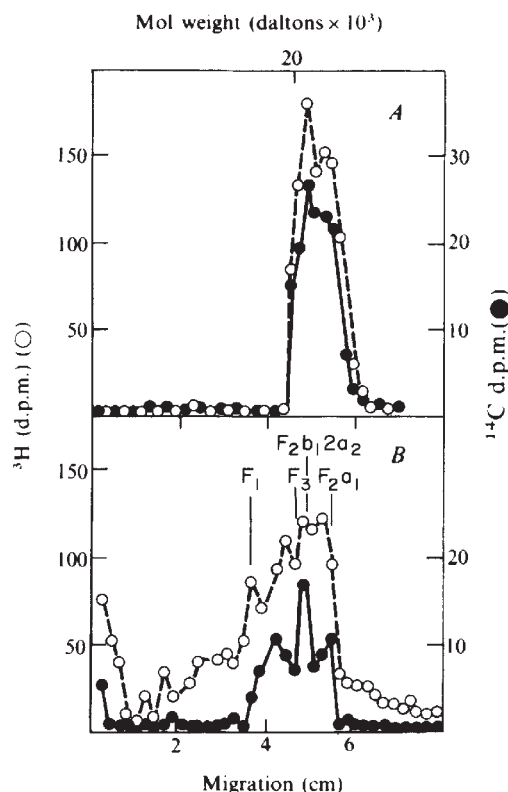


Fig. 2 Electrophoresis of histone nascent peptides extracted from acetylated polyribosomes. Nascent peptides were released with puromycin⁴ from polyribosomes acetylated *in vivo* and *in vitro*. Histone nascent peptides were extracted from the puromycin supernatants as described in the text. Samples of 100–200 µg of the dried material containing histone nascent peptides were resuspended in 1 N acetic acid, 10 N urea and 1% mercaptoethanol, and analysed by (A) sodium dodecyl sulphate-acrylamide gel electrophoresis as described in Fig. 1, or (B) urea-acrylamide gel electrophoresis at pH 2.5 (ref. 11). Symbols are as in Fig. 1. Histone standards were also run in parallel and were located by scanning the gels at wavelengths 280 nm, 600 nm, after staining with Coomassie blue⁶.

that most of the acetyl groups introduced *in vitro* were associated with the 'acidic' fraction and therefore represent N-terminal acetylation. Only 13% of the acetyl groups attached *in vivo* were found in the 'acidic' fraction. We also tried to identify the N-acetyl amino acids in the 'acidic' fractions. Analysis by paper chromatography (Fig. 3A) showed that most of the acetylated material has the same R_F as the standard N-acetylserine (10 cm). Analysis in paper electrophoresis (Fig. 3B) also shows the same mobility for the standard acetylserine and the acetyl amino acids in the 'acidic' fraction derived from the nascent peptides acetylated *in vitro*.

The experiments described here demonstrate that histone-like nascent peptides can be acetylated *in vitro* on polyribosomes from regenerating rat liver. They also show that most of the acetyl groups are attached to N-terminal amino acids, in particular to the amino group of serine. These observations can account for the *in vivo* acetylation of nascent peptides in polyribosomes from regenerating rat liver and provide direct evidence for a mechanism that can

Table 1 Dowex 50 H⁺ Fractionation of ³H-¹⁴C-Acetyl Peptides from Histones Extracted from Polysomes of Hepatectomised Rats*

Fraction	³ H d.p.m.	%	¹⁴ C d.p.m.	%
'Acidic' (N-terminal blocked)	720	13	1,470	96
'Basic' (N-terminal free)	4,470	85	57	5

* Histone nascent peptides (about 1.5 mg) extracted from polyribosomes acetylated *in vivo* and *in vitro* were resuspended in 2 ml of 0.1 M sodium bicarbonate, pH 8. Enzymatic digestion with trypsin, pronase and carboxypeptidase A and B was performed as described⁹. After removal of any cold trichloroacetic acid (TCA)-insoluble material by centrifugation and extraction of TCA from the supernatants with ether, the digests were dried under vacuum, resuspended in 2 ml of water and absorbed onto 1 × 8 cm Dowex 50 H⁺ columns. The columns were washed with 20 ml of water followed by 20 ml of 0.5 N ammonium hydroxide to obtain 'acidic' and 'basic' fractions respectively. The radioactivity in duplicate 0.2 ml aliquots from the fraction was measured by liquid scintillation counting with 10 ml of Scintisol (Isolab).

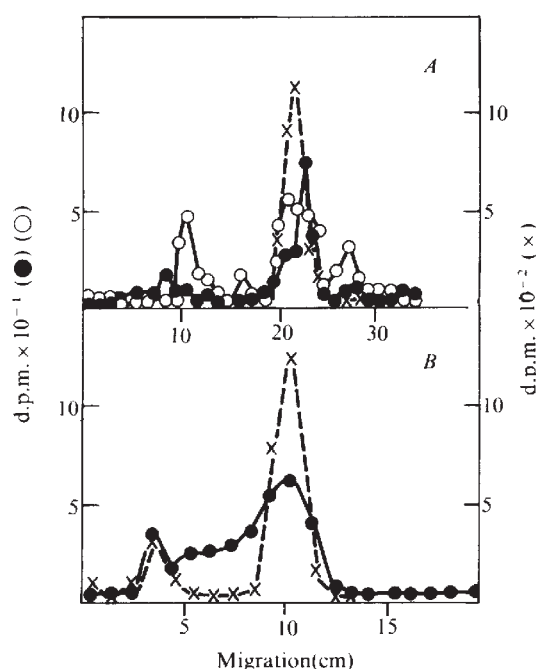


Fig. 3 Chromatographic and electrophoretic characterisation of acetyl peptides in the enzymatic digests. The water eluates from Dowex 50 H⁺ columns (see Table 1) were flash evaporated and the residue resuspended in a small volume of water. Aliquots (10 µl) were analysed by (A) paper chromatography developed with *n*-butanol:acetic acid:water (12:3:5) and (B) paper electrophoresis¹². Standard N-³H-acetylserine was prepared¹³ and included in each analysis. Paper strips (1 cm) were eluted with 1 ml of water for 1 h in counting vials, and the radioactivity was measured in a liquid scintillation counter with 10 ml of Scintisol. Solid circles and open circles represent values of nascent peptides labelled *in vitro* ¹⁴C and *in vivo* ³H, respectively. X represents N-³H-acetylserine standard.

explain the presence of N-terminal acetyl groups in liver proteins, without the necessary involvement of N-acetylation during the initiation of protein synthesis⁵. The acetyltransferase activity found in rat liver ribosomes^{6,9} may be the enzyme involved in the N-terminal acetylation of nascent peptides observed in this study. Since relatively few proteins are known to have N-acetyl termini¹, this enzyme must have a relatively high specificity. Further studies will be required to define this specificity in terms of the terminal sequence and the minimal size of the peptide substrate. Enzyme activities able to acetylate haemoglobin¹⁴ or histone¹⁵ have

been found in ribosome-free reticulocyte lysates and rat liver postmicrosomal supernatants respectively. To what extent these activities are functionally involved in the N-terminal acetylation of proteins deserves further study.

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Spontaneous Re-formation of a Broken Peptide Chain

BASIC pancreatic trypsin inhibitor (BPTI) is a protein of fifty-eight amino acid residues, which inhibits the serine proteinases trypsin, chymotrypsin, plasmin and kallikrein. The sequence has been fully elucidated¹ (Fig. 1) and the three-dimensional

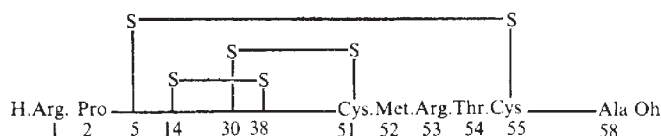


Fig. 1 BPTI part structure.

structure has been determined to high resolution by X-ray crystallography^{2,3}. In connection with studies on the partial synthesis of analogues of BPTI for the investigation of protein folding, we have examined in detail cleavage of the protein by cyanogen bromide⁴. We have found that the chain-cleaved product is unstable, and that on standing it is spontaneously transformed into an analogue of BPTI containing an intact chain of fifty-eight amino acid residues.

The single methionine residue in BPTI occurs at position 52 of the peptide chain. The crystal structure indicates that it is approximately in the middle of the only extended region of α -helix, and lies near the surface of the tightly folded molecule. Chemical evidence⁵ indicates that the methionyl side chain is accessible to some, but not all, modifying reagents when the molecule is in its native conformation. There are also six cysteinyl residues in BPTI, which form three disulphide bridges. Two of these residues also occur in the α -helical region, at positions 51 and 55, straddling the position of the methionine residue. Thus, when the peptide chain of the native molecule has been broken with conversion of the methionine to homoserine lactone by cyanogen bromide, the resulting two

backbone fragments are still held together by a disulphide linkage (between residues 5 and 55). As we shall demonstrate, this covalent bond between the fragments promotes the resynthesis of a peptide bond between residues 52 and 53 by attack of the newly generated amino terminus on the lactone carbonyl.

Treatment of BPTI with a hundred-fold excess of cyanogen bromide caused conversion of methionine to homoserine in 80% of the molecules within 20 h. Only one of the various expected products of cyanogen bromide treatment, the species in which chain cleavage between residues 52 and 53 has occurred and in which the homoserine residue is in the lactone form, should differ in net charge at neutral pH from BPTI. Accordingly ion-exchange chromatography of the cyanogen bromide-treated protein resolved two major peaks (Fig. 2). The first peak emerged at the same position as native BPTI; amino acid analysis of this material indicated that it contained

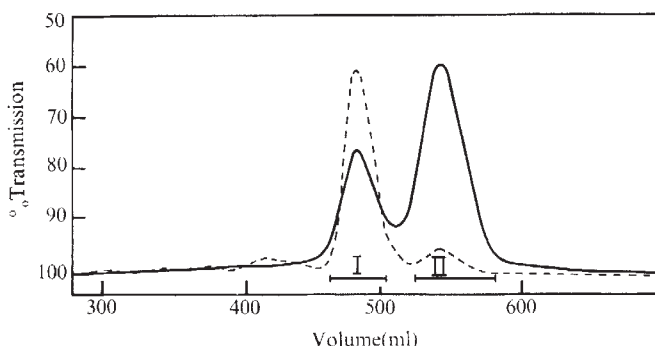


Fig. 2 (—), Ion-exchange chromatogram on a 1.3×30 cm column of carboxymethylcellulose (CM-52) of the desalted product from treatment of native BPTI with a hundred-fold excess of CNBr in 70% formic acid. The protein was chromatographed at pH 6.2 in 0.01 M phosphate buffer using a linear gradient of NaCl^{6,7} (0–0.5 M, 400 ml in each chamber.) The position of peak I is identical to that obtained for native BPTI. Peak II corresponds to a more positive species. (---), Rechromatography of a sample of II after 6 d at pH 6.5.

all of the molecules with residual methionine. The second peak contained protein (II) in which the peptide chain has been broken. No methionine could be detected in this product, but acidic hydrolysates which had been heated in pyridine acetate just before analysis⁸ contained 1.0 residue of homoserine per protein molecule. Furthermore, the new N-terminus, arginine 53, could be released in high yield by digestion of II with leucine aminopeptidase⁹. Although the normal N terminus of native BPTI is also arginine, this residue is not released by leucine aminopeptidase, presumably because residue 2 of the protein is proline.

Tryptic maps of II also definitely established backbone cleavage. When BPTI was reduced and carboxymethylated with ¹⁴C-labelled iodoacetic acid, the resulting product was readily digested by trypsin^{10,11}, and electrophoresis at pH 6.5 resolved the four radiolabelled peptides (Fig. 3a). Two of these contained two carboxymethylcysteine residues each: peptide 1–15 (including cysteine residues 5 and 14) and peptide 27–39 (including cysteine residues 30 and 38). The other two peptides, 47–53 and 54–58, contained only one radiolabelled residue each, numbers 51 and 55, respectively. When II was reduced and carboxymethylated in the same manner, the hexapeptide 53–58 was removed upon desalting through Sephadex G25. Tryptic digestion of the large protein product and subsequent electrophoresis showed that the normal peptides 47–53 and 54–58 were virtually absent, while a new, more acidic peptide was present (Fig. 3b). This new peptide had an amino acid composition consistent with sequence 47–52 (52=homoserine).

The properties of II have been observed to change with time in a manner which indicates resynthesis of the peptide backbone. This process has been primarily studied through the tryptic peptides of ^{14}C -carboxymethylated material. These studies were made quantitative by adding uniformly ^3H -carboxymethylated BPTI to samples before digestion, to act as an internal control. The ratio of ^{14}C to ^3H in the peptides 47-53 and 54-58 relative to that in the peptides 1-15 and 27-39 indicates the fraction of material in which the 52-53 peptide bond has been re-formed. For preparations of II in which the ion-exchange chromatography was performed at room temperature and the products were left at $\text{pH} \sim 6.2$ for a few hours, the amount of resynthesised protein varied from 15 to 35%. When chromatography was performed at 4°C and the products were trapped at approximately $\text{pH} 3$ by pre-charging each fraction with a small amount of acetic acid, the concentrations of peptides 47-53 and 54-58 detected could be kept to 5%.

The observations above are fully consistent with spontaneous resynthesis of the peptide chain in neutral solutions, and we have been able to follow the course of the resynthesis reaction with this radiolabelling procedure. A solution of II kept at $\text{pH} 6.3$ was sampled over the course of 2 d, and the described mapping procedure was employed at each stage. There was a progressive increase in the relative proportion of the peptides 47-53 and 54-58 and a corresponding attenuation in the amount of peptide 47-52 detected in successive samples, thereby indicating clearly the reconstitution of the protein backbone with time. Quantitative analysis of these successive samples by the method of double-counting outlined previously indicated an initial half-life for the resynthesis reaction of about 30 h. Similar analysis of a sample of II which had been reduced and carboxymethylated after standing 4 d at $\text{pH} 6.3$ (Fig. 3c) indicated the presence of peptides 47-53 and 54-58 in at least 85% yield and only a small amount of peptide 47-52. When the product obtained from such a resynthesis reaction was rechromatographed on the ion-exchange column used to isolate II, the majority of the material emerged in the position corresponding to BPTI (Fig. 2).

Neutral solutions of II also gained in inhibitory potency as a function of time. Preparations of II were found to be only 5% as potent as native BPTI in inhibiting trypsin (as determined by the benzoylarginine *p*-nitroanilide assay)¹² and 30% to 50% as potent in inhibiting chymotrypsin (as determined by the acetyltryptophan ethyl ester assay)¹³. In both cases the rate of increase towards the level of activity of BPTI agreed well with the rate of resynthesis demonstrated by the radiolabelling experiments.

The resynthesised protein, [52-homoserine]-BPTI, has been found to possess between 85% and 95% of the inhibitory potency of BPTI towards both trypsin and chymotrypsin. Furthermore, in the acetyltryptophan ethyl ester assay, in which BPTI is slowly displaced from chymotrypsin by the substrate, solutions of [52-homoserine]-BPTI of equal initial inhibitory potency exhibited a rate of inhibitor displacement indistinguishable from that for BPTI. The binding constants to chymotrypsin for both molecules must therefore be virtually identical. This is not surprising, as residue 52 is far removed from the active site of the molecule¹¹, and oxidation of methionine-52 has previously been shown not to alter its inhibitory properties⁵.

Experiments involving reduction of the disulphide bridges and their subsequent reoxidation also demonstrated the resynthesis of the protein backbone in II. Native BPTI, which had been fully reduced in strong denaturing agents, could be reoxidised in neutral pH buffers by air or disulphide reagents with full regain of inhibitory activity. This was not true of II, which after reduction would regain activity only to the extent to which there was resynthesised material present. Nor would such samples regain activity beyond the level indicated when a ten-fold excess of the cleaved hexapeptide was added to the

oxidising solution. Purified samples of [52-homoserine]-BPTI, however, were readily reoxidised to a fully active state after reduction. The rate of reoxidation of reduced [52-homoserine]-BPTI by disulphide reagents, measured as its rate in regaining potency as a trypsin inhibitor, was comparable to that measured simultaneously for reduced BPTI.

Our discovery of an example of facile resynthesis of a peptide backbone in a cyanogen bromide-cleaved protein indicates that this may be a practical route to the partial synthesis of some protein analogues. The direct coupling of lactone and amino components under favourable circumstances, as in this case where the fragments are held close together by a disulphide bridge, may provide routes for partial synthesis complementary to those already suggested employing such lactone functions¹⁴, but which require more lengthy synthetic routes. We are at present exploring methods of utilising this more direct resynthesis reaction *en route* to new analogues of BPTI.

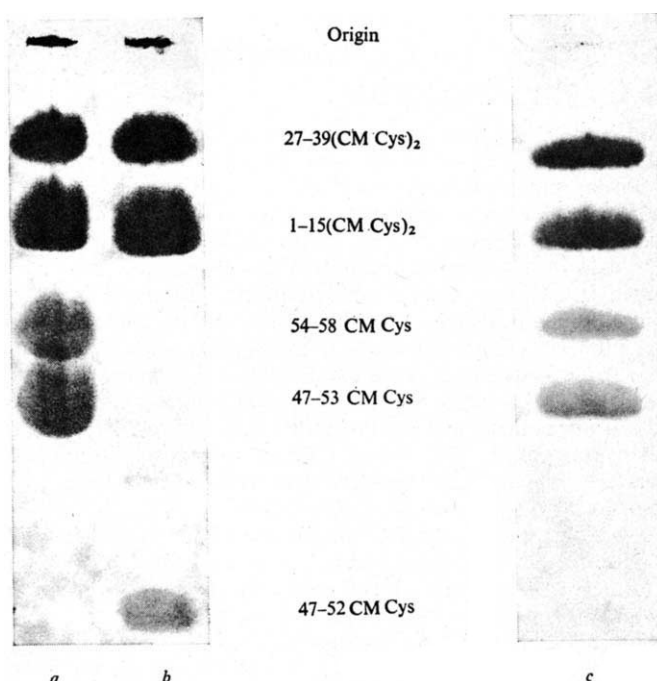


Fig. 3 Electrophoresis maps at $\text{pH} 6.5$ of the tryptic digests of ^{14}C -carboxymethylated samples of, a, BPTI, b, II, and of c, the protein isolated from an aqueous solution of II after 4 d at $\text{pH} 6.5$. These proteins were reduced in 6 M guanidinium hydrochloride at $\text{pH} 8.1$ with 20 mM dithiothreitol, and carboxymethylated by addition of a slight (10%) excess of ^{14}C -labelled iodacetate. After desalting on Sephadex G25, trypsin digestions were performed in 0.1 M ammonium bicarbonate for 2-3 h at 25°C . The identity of the radioactive peptides has been established by quantitative amino acid analysis.

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Structure and Conformation of a Cyclic Tripeptide

MANY cyclic peptides have interesting biological functions and the details of their molecular structure and conformation have been the subject of extensive investigations. Cyclic dipeptides such as diketopiperazine have been synthesised and shown to occur with the peptide units in the *cis* configuration^{1,2}. In the case of a tripeptide, cyclisation can take place only if all three units are in the *cis* configuration³. In cyclic peptides with four units also, *cis* peptides are found^{4,5}. As the number of the peptide units increases, the more stable *trans* configuration is generally more common^{6,7}. We report here the main results of our X-ray crystallographic investigations of the cyclic tripeptides L-Pro-L-Pro-L-Pro and L-Pro-L-Pro-L-Hyp (hereafter called CTP 1 and CTP 2, respectively). CTP 1 was synthesised by Rothe *et al.*⁸ and its derivatives have been prepared by Blout and his collaborators⁹.

Venkatachalam³ predicted that in the cyclic tripeptide CTP 1 all three peptides should be in the *cis* configuration for ring closure to be achieved. In his study, however, the conformational parameters were worked out on the assumption that the proline rings and the nitrogen environment are essentially planar. Nuclear magnetic resonance (NMR) studies on cyclic peptide derivatives have shown that the peptide units are indeed *cis* planar, but the proline rings are non-planar with the nitrogen atom out of the mean plane through the remaining four atoms of the proline ring⁹. Our studies show that the peptide units are *cis* planar in general, but the conformation of the proline rings is somewhat different from that reported in the NMR studies.

Crystals of CTP 1 and CTP 2 were recrystallised from material given to us by Professor E. R. Blout of Harvard Medical School. The crystal data are shown in Table 1. The diffraction data were collected manually on a GE XRD-3 diffractometer to a limit of $2\theta = 150^\circ$. These two acentric structures, with 42 and 22 non-hydrogen atoms respectively in the asymmetric units, were solved by the direct methods using the MULTAN program¹⁰, and refined by the block diagonal least squares method. During the refinement of CTP 2, it became apparent that the hydroxyl group was disordered. The electron density difference map showed two positions OH (1) and OH (2) for the hydroxyl group, the relative occupancy of OH (2) being twice the other. It is interesting that, for both these sites, the OH group is linked by a strong hydrogen bond (2.7 Å) to the carbonyl oxygen of a neighbouring molecule. All the hydrogen positions in the molecule of CTP 2 were established from the electron density difference map. The diffraction data from CTP 1 were of poor quality, and consequently unambiguous location of the hydrogen atoms was not possible in this case. A view of the molecule of the tripeptide as it occurs in the crystal structure of CTP 2 is shown in Fig. 1.

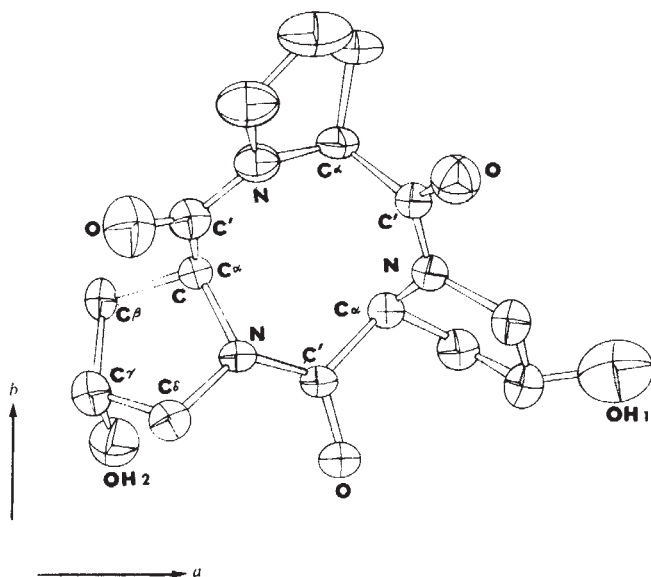


Fig. 1 View of the tripeptide molecule in CTP 2 looking down the *c* axis¹³.

Table 1 Crystal Data of CTP 1 and CTP 2

	CTP 1	CTP 2
<i>a</i>	15.95 Å	16.28 Å
<i>b</i>	19.09 Å	9.45 Å
<i>c</i>	9.23 Å	9.23 Å
<i>z</i>	8	4
Space group	P 2 ₁ 2 ₁ 2 ₁	P 2 ₁ 2 ₁ 2 ₁
λ	1.5418 Å	1.5418 Å
Number of measured reflections	3,299	1,762
Percentage used for electron density calculation	79	97
Reliability index	0.13	0.06

The orientation of the tripeptide molecules in the structures of CTP 1 and CTP 2 is very similar when viewed down the *c* axis. The two molecules (*A* and *B*) in the asymmetric unit of CTP 1 are inclined to each other by about 55° . The average torsion angle $\chi_1(\text{N}-\text{C}_\alpha-\text{C}_\beta-\text{C}_\gamma)$ for the eight residues was 31° , in good agreement with the value obtained from NMR data⁹. The puckering of the different proline residues in the three tripeptide molecules is given in Table 2. The average value of the torsion angle $\omega(\text{C}_2-\text{C}'-\text{N}-\text{C}_2)$ is 1.8° for eight of the nine residues, while in the hydroxyproline residue it is as high as 19° . These torsion angles showed a fairly wide spread of about 5° . Therefore it seems that the cyclisation of three peptides could be achieved without invoking any excessive non-planarity of the peptide group. The backbone dihedral angles ϕ and ψ range from -94° to -110° and 83° to 98° respectively. These are appreciably different from the values -78° and 109° predicted earlier³. Because of these variations in the torsion angles, from peptide to peptide in the tripeptide molecule, the backbone conformation in the crystal does not possess a true three-fold symmetry, assumed in interpreting the results of the solution studies⁹. Thus, we find many new types of conformation for the proline rings here different from any of those reported before in X-ray and NMR studies¹¹.

We have averaged the latest data from eight crystallographically independent *cis* proline residues in the crystals of CTP 1 and CTP 2 and obtained the dimensions of the standard *cis* proline residue (Fig. 2). The average ring torsion angles $\chi_1(\text{N}-\text{C}_\alpha-\text{C}_\beta-\text{C}_\gamma)$, $\chi_2(\text{C}_\alpha-\text{C}_\beta-\text{C}_\gamma-\text{C}_\delta)$, $\chi_3(\text{C}_\alpha-\text{C}_\beta-\text{C}_\delta-\text{N})$, $\chi_4(\text{C}_\gamma-\text{C}_\delta-$

Table 2 Puckering of Proline Residues in the Three Tripeptide Molecules

Atom out of plane	CTP 1(A)	No. of residues CTP 1(B)	CTP 2
C _α	1	—	1
C _β	2	3	1
N	—	—	1

N-C_α) are also shown in Fig. 2. It is worth noting that in CTP 1 and CTP 2 the C'-N bond distance (1.362 ± 0.015 Å) is significantly larger than the value (1.32 Å) found in the *trans* peptide unit postulated by Corey and Pauling¹². The average parameters we have obtained agree with the values reported by Karle².

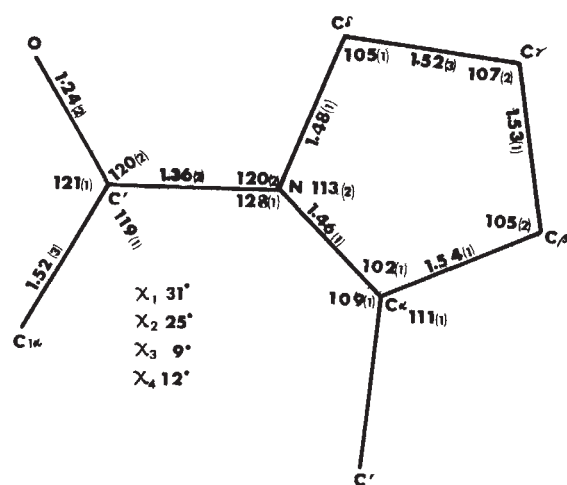


Fig. 2 Dimensions of the standard *cis* proline residue averaged from eight independent residues in CTP 1 and CTP 2. The RMS deviation in the dimensions in the eight residues is also indicated.

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Interferonogenicity and Antigen Recognition

Not only viral and syngeneic polynucleotides but also bacterial endotoxins, PHA (refs 1 and 2), heterologous and, in certain cases, homologous gammaglobulins can induce interferon³⁻⁵. The question arises whether or not antigenicity correlates with interferonogenic properties. We therefore studied the interferonogenic properties of syngeneic and allogeneic living or irradiated mouse spleen cells (SC), xenogeneic and allogeneic erythrocytes and syngeneic materials (erythrocytes and sera) that had become autoantigenic after treatment with trypsin, iodination or heating.

SC were obtained from aseptically removed mouse spleens by soft compression in the glass Potter homogeniser. The cells were twice washed with medium 199 by centrifuging at 2,000 r.p.m. for 15 min, and the number of living nucleated cells was counted. Iodinated serum proteins were prepared according to Wormald⁶. The activity of serum interferon

Table 1 Interferonogenicity of Normal SC and Irradiated SC (ISC) for Syngeneic and Allogeneic Recipients*

Experiment No.	Recipient mice	Material injected	Time after injection (h)	Interferon activity† (units ml ⁻¹)
1	DBA/2	SC-Balb/c	6	0
	DBA/2	SC-Balb/c	12	60
	DBA/2	SC-Balb/c	24	50
	DBA/2	SC-Balb/c	48	20
	DBA/2	SC-DBA/2	6	0
	DBA/2	SC-DBA/2	12	0
	DBA/2	SC-DBA/2	24	0
	Balb/c	SC-Balb/c	12	0
	Balb/c	Without injection	12	0
2	F ₁ (CBA × C57Bl/6j)	SC-F ₁ (CBA × C57Bl/6j)	12	0
	F ₁ (CBA × C57Bl/6j)	SC-C57Bl/6j	12	5
	F ₁ (CBA × C57Bl/6j)	ISC-C57Bl/6j	12	0
	F ₁ (CBA × C57Bl/6j)	Without injection	12	0
3	C57Bl/6j	SC-Balb/c	16	70
	C57Bl/6j	SC-Balb/c	43	40
	C57Bl/6j	ISC-Balb/c	16	30
	C57Bl/6j	ISC-Balb/c	43	10
	C57Bl/6j	SC-Balb/c	16	20
	irradiated*			
	C57Bl/6j	SC-Balb/c	43	0
	irradiated			
	C57Bl/6j	ISC-Balb/c	16	5
	irradiated			
	C57Bl/6j	ISC-Balb/c	43	0
	irradiated			
4	C57Bl/6j	SC-DBA/2	4	10
	C57Bl/6j	SC-DBA/2	17	40
	C57Bl/6j	ISC-DBA/2	4	5
	C57Bl/6j	ISC-DBA/2	17	30

* SC exposed to gamma-irradiation, total dose 16,000 r, and mice recipients C57Bl/6j (in experiment 3), in a dose (total) of 850 r.

† Unit of interferon activity is a reciprocal of the endpoint serum interferon dilution inhibiting cytopathic effect.

was evaluated by a standard method⁷: 1 ml of the corresponding dilution of the serum to be studied (interferon) was placed on 48 h cultures of mouse L cells in each tube. The serum was removed 18–24 h later and 100 tissue culture pathogenic dose 50% (TCPD₅₀) of vesicular stomatitis virus were added and 24 h later the highest dilution of interferon that inhibits the cytopathic effect by 50% was determined. The tested dilutions of mouse sera higher than 1:20 were not toxic to L cells. In each experiment the interferon was titrated repeatedly two or three times. Mice of the strains Balb/c, DBA/2, C57Bl/6j and hybrids F₁ (CBA×C57Bl/6j) were used. Groups of fifteen to twenty-five mice recipients were inoculated with the following antigens: 10⁶ of SC in 0.8 ml of medium 199, 6×10⁹ erythrocytes in 0.5 ml of medium 199 and sera treated in different ways, in a volume of 0.5 ml. At different times (from 3 to 48 h) after antigen injection blood was taken from the mouse retro-orbital sinus and interferon was determined in the serum.

Intravenous injection of allogeneic SC leads to a marked interferon production in mice (Table 1). The maximum interferon concentration in the serum of mice recipients was observed 12–24 h after SC injection. Such interferon production takes place even in cases in which a donor and a recipient differ from each other in weak histocompatibility

antibody formation and interferon production in parallel¹. Green *et al.*¹³ suggested that immune recognition mechanisms are involved in enhanced interferon production in secondary immune response to non-viral antigens.

The experimental evidence presented above shows that cells and substances immunologically recognisable as 'self' do not induce interferon production and conversely cells and substances recognisable as 'non-self' do induce interferonogenesis after primary administration. The quantities of interferon produced after injection of various antigens were different. We suggest that antigen induces interferon production in cells involved in the first stage of the immune response.

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Table 2 Interferon Activity after Injection into Mice of Normal and Trypsin-treated Red Blood Cells (RBC) and Iodinated and Heated Sera

Experiment No.	Recipient mice	Material injected	Time after injection (h)		
			6	12	24
5	C57Bl/6j	RBC-C57Bl/6j	0	0	0
		RBC-Balb/c	5	5	5
6	DBA/2	RBC-Balb/c	—	0	—
		RBC-DBA/2	—	0	—
7	C57Bl/6j	RBC-sheep	5	5	5
	C57Bl/6j	Trypsin-treated RBC-C57Bl/6j	5	10	20
	C57Bl/6j	C57Bl/6j serum	—	—	0
	F ₁ (CBA×C57Bl/6j)	Syngeneic serum	—	0	—
F ₁ (CBA×C57Bl/6j)	F ₁ (CBA×C57Bl/6j)	Iodinated syngeneic serum	—	20	—
		Syngeneic serum heated at 56°	—	5	—

loci: mouse strains DBA/2 and Balb/c (experiment 1, Table 1). An intravenous injection of syngeneic SC does not lead to interferonogenesis. From experiments 2 and 3 with F₁ hybrids and with irradiated SC and/or irradiated recipients (see Table 1), it is evident that both donor and recipient lymphoid cells produce interferon.

This serum interferon was stable at 24 h at pH 2, lost antiviral activity in the presence of 0.1% of trypsin at 37° C for 1 h, failed to sediment at 100,000g for 1 h, did not lose activity when cultures treated with the serum interferon were washed before virus challenge, was active in mouse L cells against Venezuelan equine encephalitis virus and Sindbis virus, and did not possess any antiviral properties in the continuous cell culture of human amniotic F1 cells.

Allogeneic and xenogeneic erythrocytes induce only slight interferon production. Non-interferonogenic syngeneic erythrocytes acquire interferonogenic activity when treated with trypsin (Table 2). Syngeneic mouse serum is non-interferonogenic. The same serum becomes interferonogenic after heating or iodination.

In this laboratory the role of interferon in lymphocyte-target cell interaction has been established^{8–11}. Interferon induces the sensitivity of target cells to the cytotoxic effects of normal allogeneic lymphocytes¹². Other data on the connection of immune response and interferonogenesis have accumulated. Lymphoid cells were established as the main interferon producers *in vivo*^{1,2}. A variety of agents suppressed

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Spontaneous Fusion of Rat Liver Lysosomes *in vitro*

THE fusion of membranes is a key to the control of lysosomal function. Membrane fusion is involved in both the intra- and extracellular digestion of extracellular material (fusion between endocytotic vacuoles or between plasma membrane and enzyme-bearing vesicles) as well as in the formation of autophagic vacuoles. Primary lysosomes fuse readily with newly formed endocytotic vacuoles^{1,2}; they seem to be able to fuse with heterolysosomes³ but never directly with mitochondria or nuclei. Because of scanty information the apparent specificity in the fusion pattern of primary lysosomes cannot be explained in terms of relationships between membrane composition and function. Dingle⁴ has proposed, in analogy to the coalescence of closed shells of fluid, or the 'breaking' of an emulsion by agglomeration of droplets to larger ones, that the fusion of cell membranes of high surface tension will be thermodynamically favoured. According to this suggestion lysosomes should be regarded as high surface tension vesicles.

The above considerations prompted us to investigate the possibility of spontaneous fusion of a lysosomal population *in vitro*. Lysosome-rich suspensions were incubated at 0° C and at 37° C for time intervals extending up to 3.5 h. At zero time, 0.5, 1 and 3.5 h the suspensions were analysed for free and 'available' acid phosphatase activity (Fig. 1), and

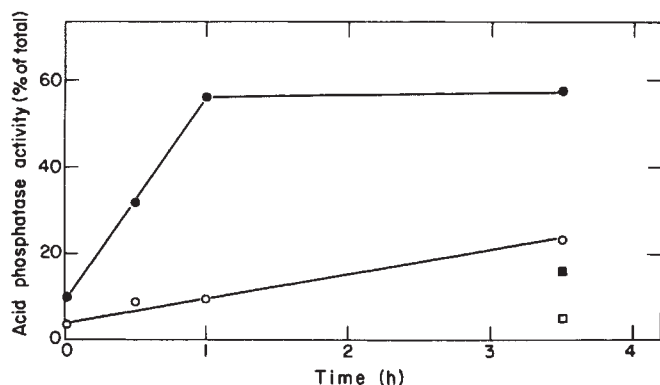


Fig. 1 Time dependence of free (○—○) and available (●—●) acid phosphatase activity in lysosomal suspensions incubated at 37° C. Corresponding values obtained in lysosomal suspensions incubated for 3.5 h at 0° C are also included (□, ■). Incubation mixture consisted of 23.5 mg of lysosomal protein ml⁻¹ isolated as described¹³, 0.25 M sucrose and 0.01 M Na-HEPES, pH 7. At the specified time intervals, 20 µl aliquots were removed from the suspensions and assayed¹⁴ with *p*-nitrophenyl phosphate for free activity (detected in the medium after sedimentation of lysosomes), available activity (exhibited by the whole suspension), and total activity (reaction mixture made 0.1% in Triton X-100).

were fixed for electron microscopic examination. Continuous slow release of acid phosphatase to the medium was observed in lysosomal suspensions incubated at 37° C (20% in 3.5 h); no such release was observed in suspensions incubated at zero degrees. The latency of acid phosphatase activity was, however, markedly affected during incubation at 37° C. After about 1 h of incubation the available acid phosphatase activity of lysosomal suspensions increased from a value of 10% to about 60% of the total activity. The lysosomal membranes thus seem to have undergone a change in their barrier properties towards *p*-nitrophenyl phosphate, a substrate for acid phosphatase. No such change was observed in suspensions incubated at 0° C.

Electron micrographs of zero time suspensions showed populations of vesicles mostly regular in shape: spherical or ellipsoid. Some of the vesicles were more phosphotungstic acid positive, others were stained only in the limiting membrane and its close periphery (Fig. 2a). The same pattern was obtained in a histochemical assay of acid phosphatase (Fig. 2b). The dramatic change in both the dimensions and the shape of the suspended vesicles as a result of a 3.5 h incubation period at 37° C is shown in Fig. 2c. The average diameter of lysosomes incubated for 3.5 h at 37° C was twice that obtained for zero time control lysosomes (~0.5 µm). This indicates roughly at least a four-fold increase in vesicular surface area. Figure 2d shows lysosomes incubated for 3.5 h at zero degrees. It thus seems that the morphological changes are temperature dependent.

It is worth noting that lysosomal suspensions kept at 0° C (Fig. 2a and d) contain a significant percentage of mitochondria. No mitochondria could be detected in pellets of lysosomes incubated for 3.5 h at 37° C; suggesting mitochondrial breakdown, possibly due to the presence of free lysosomal enzymes in the medium.

The enlargement of vesicular contours could be brought about either by intralysosomal degradation of macromolecules, leading to an increase in osmotic pressure and lysosomal swelling or by fusion of vesicles. The first mechanism would imply that the lysosomal membrane has an unusual expanding capacity, since lysosomes are not endowed with biosynthetic capacity. Measurements of membrane thickness in both 0° C control and 37° C incubated lysosomes do not support such a mechanism. Fusion of vesicles during the incubation period at 37° C is the most plausible explana-

tion for the morphological transformation of the lysosomal suspension. Since 3.5 h incubation yielded a rather homogeneous population of enlarged vesicles, it could be reasoned that on shorter incubation intermediate steps in the fusion process could be arrested. Figure 3 shows such intermediate steps. Note the small protrusions which develop at areas of

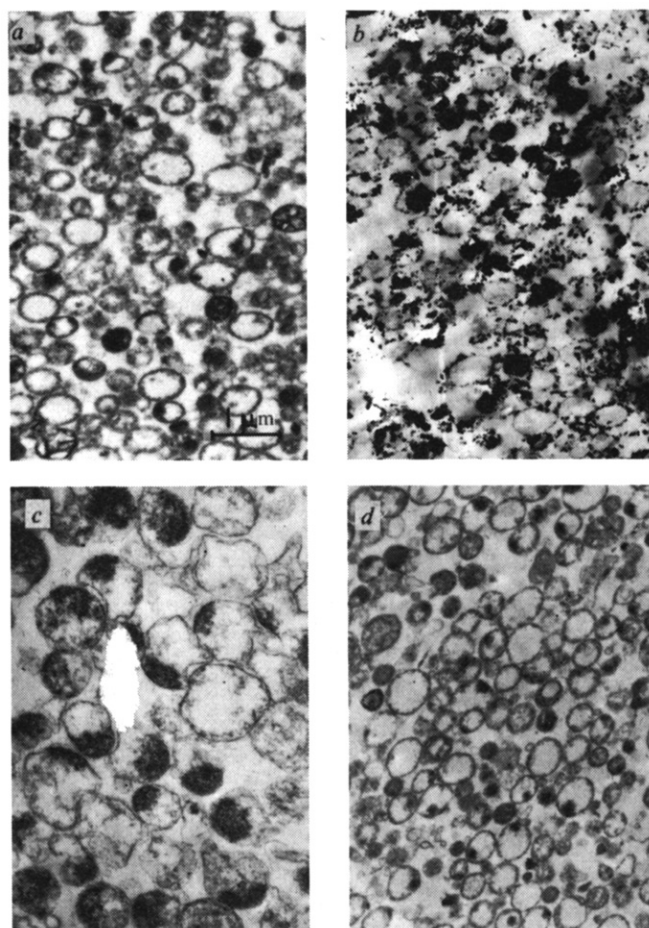


Fig. 2 Characteristic morphology of lysosome-rich suspensions, ×8,530. *a*, Zero time suspension; *b*, zero time suspension stained histochemically for acid phosphatase¹⁵; *c* and *d*, lysosomal suspensions incubated for 3.5 h at 37° C and at 0° C, respectively. Incubation of the lysosomal suspensions (described in Fig. 1) was terminated by centrifugation. The lysosomal pellets were resuspended in 0.1 M Na-cacodylate, pH 7.4, containing 2% of glutaraldehyde. After 1 h of fixation the pellets were washed, and post-fixed for 1 h with 1% osmium tetroxide. The resulting precipitate was dehydrated and embedded in epoxy resin according to Spurr¹⁶. Thin sections obtained with Sorvall, Porter-Blum MT 2-B ultra-microtome were stained for 30 s with 1% phosphotungstic acid in 70% ethyl alcohol, and were analysed in a Phillips 300 electron microscope.

close contact between lysosomes (Fig. 3a and b). It would seem reasonable that these protrusions facilitate the interdigitation of membrane elements of two vesicles. A latter step involving membrane fusion would establish permanent connections between vesicles. The resulting structures seem to be closed vesicles encompassed by an intact membrane. Phosphotungstic acid positive material tends to aggregate at one pole of the vesicles (Fig. 2c). As previously pointed out the observed two-fold increase in particle diameter indicates for a sphere a four-fold increase in surface area and an eight-fold increase in volume. If it is assumed that the total membrane area is constant and that intralysosomal volume is preserved (no swelling following fusion), then it is to be expected that the vesicles resulting from lysosomal

fusion would not be spheres. This indeed seems to be the case (see Fig. 2c). The continuous release of acid phosphatase to the suspending medium (Fig. 1) suggests that some lysis accompanies lysosomal fusion. Such a process has been proposed as a prerequisite to chemical^{5,6} and viral⁷ induced fusion of cells.

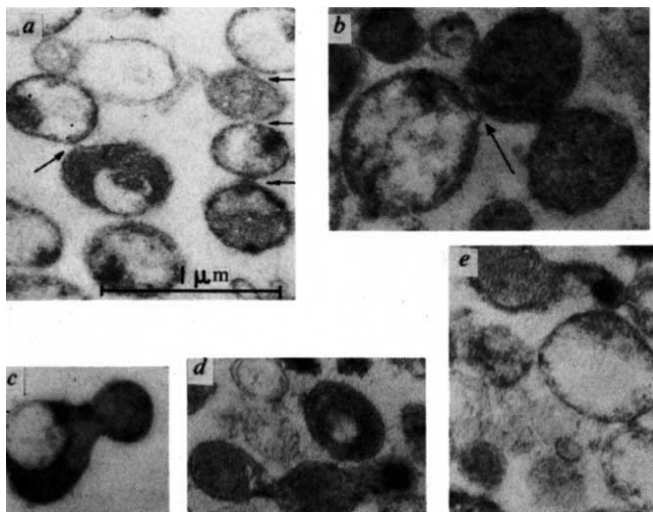


Fig. 3 Intermediate steps in lysosome fusion. Lysosomal suspensions (details in legend to Fig. 1) were incubated for 0.5 h (a and c) and for 1 h (b, d, e) at 37° C. Incubation was terminated by centrifugation and the pellets processed as described for Fig. 2. a, $\times 22,500$; b, c, $\times 67,500$; d, e, $\times 29,160$.

The observed temperature dependence of fusion could be attributed to the involvement of enzyme action, though chemically induced cell fusion⁸ has also been shown to be markedly temperature dependent.

The molecular events preceding fusion are not yet understood. It seems, however, that extraorganelle energy sources or bivalent cations are not essential elements in the process. It has been suggested⁹ that a primary requirement for the fusion of two biological membranes is that both membranes involved have a relatively high proportion of their phospholipid molecules in the micellar configuration. It was also argued that molecules like lysolecithin, found in lysosomal membrane phospholipids⁴, can induce micellar configuration⁹. Moreover, the presence of phospholipases in lysosomes which could degrade lecithin to lysolecithin has been reported^{10,11}.

Similar morphological changes, that is, the appearance of protrusions leading to bridge formation, have been reported in the case of fusion of rat parotid secretory granules with the cell membrane at the lumen¹² and in chemically induced fusion of avian erythrocytes⁵.

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Selective Binding Properties of Vitamin D Transport Protein in Chick Plasma *in vitro*

WHILE vitamin D₂ and vitamin D₃ are equipotent antirachitic agents in most mammals¹ ergocalciferol (D₂) is approximately 1/100 as potent as cholecalciferol (D₃) in preventing or curing rickets in young chicks². Although the basis of the insensitivity in chicks to D₂ has not yet been elucidated, it had been noted that radioactive vitamin D₂ is cleared from blood more rapidly than radioactive D₃ while inactive metabolites of D₂ are excreted more rapidly in the bile and faeces³. We have shown that a specific transport protein for vitamin D and D metabolites exists in the plasma of D-deficient chicks as well as in the plasma of several mammalian species⁴. These specific transport proteins serve to solubilise vitamin D compounds in the aqueous environment of plasma and to protect the steroids from oxidative inactivation⁵. Since vitamin D requires efficient plasma transport to the liver and the kidney for successive enzymatic hydroxylations⁶⁻⁸ and the 'activated' metabolites must, in turn, be transported efficiently to the target tissues, we undertook studies to test the hypothesis that the lower potency of D₂ might, in part, reflect weaker binding of vitamin D₂ and its metabolites to the chick plasma transport protein.

Our approach to this problem involved the use of D₂ and D₃ analogues to competitively displace ³H-25-OH-D₃ from the specific binding protein in plasma from various species⁹. It was necessary to first remove all non-specific binding proteins such as chylomicra, low density and very low density lipoproteins from the plasma through selective precipitation with heparin and manganese chloride¹⁰. The plasma was then diluted with barbital acetate buffer, pH 8.6 (ref. 9), and used in an assay system which included ³H-25-OH-D₃ and the vitamin D analogue to be tested. Following a 2 h incubation at 4° C, separation of the unbound ³H-25-OH-D₃ from that associated with the specific binding protein was accomplished with dextran-coated charcoal and the ³H in the supernatant was counted in Aquasol (New England Nuclear). Increasing amounts of each analogue tested produced progressive reduction of the ³H-25-OH-D₃ in the 'bound' supernatant fraction allowing the development of displacement curves and comparison of the relative binding of the D₂ and D₃ analogues by the plasma from chick and other species.

In the assay system with rat plasma, increasing amounts of vitamin D₂ and D₃ produced similar reductions of the ³H-25-OH-D₃ in the supernatant fraction (Fig. 1a) indicating equivalent binding of D₂ and D₃ by the rat transport protein. Increasing amounts of 25-OH-D₂ and 25-OH-D₃ also produced quantitatively equivalent reduction of ³H-25-OH-D₃ confirming the equivalent binding of D₂ and D₃ by the rat binding protein for vitamin D (Fig. 1b). Equivalence of binding of D₂ and D₃ was also found with the transport proteins in human, rabbit, cow, and dog plasma, species in which D₂ and D₃ are biologically equivalent¹. In contrast, using the assay

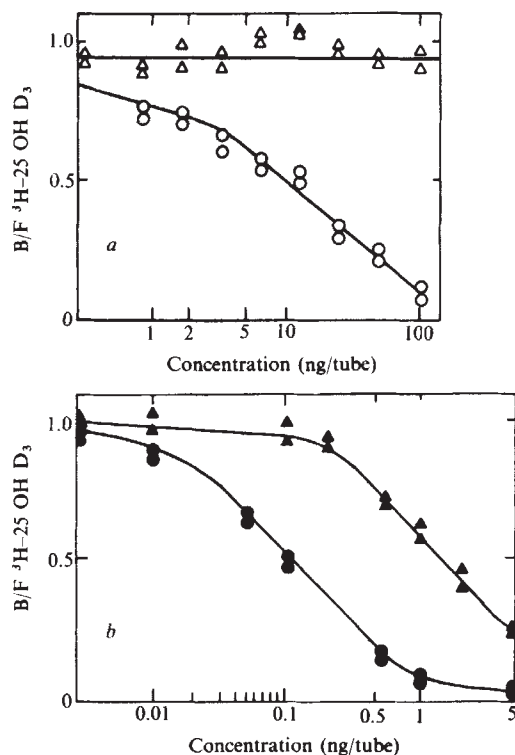


Fig. 1 Displacement of ^3H -25-OH-vitamin D_3 from D-deficient rat plasma with vitamin D analogues. Plasma obtained from D-deficient rats was cleared of non-specific binding proteins¹⁰ and diluted with barbital acetate buffer, pH 8.6. Increasing amounts of the analogue to be tested with a fixed amount of ^3H -25-OH- D_3 , all in 95% ethanol, were added to the diluted binding protein (B) or to buffer only (D). An additional aliquot of the analogue with the ^3H -25-OH- D_3 was added to a counting vial for determination of the c.p.m. added to each tube (T-total c.p.m.). Separation of unbound ^3H -25-OH- D_3 in both B and D tubes was accomplished with dextran-coated charcoal (0.25% Norit A Charcoal and 0.025% Dextran T80). Specific binding was determined by subtracting c.p.m. in the supernatant in the absence of binding protein (D) from the c.p.m. with the binding protein present (B) and the bound/free (B/F) ratio calculated: $\text{B/F} = \text{B} - \text{D} / \text{T} - (\text{B} - \text{D})$. $\circ$, D_3 ; $\triangle$, D_2 ; $\bullet$, 25-OH- D_3 ; $\blacktriangle$, 25-OH- D_2 .

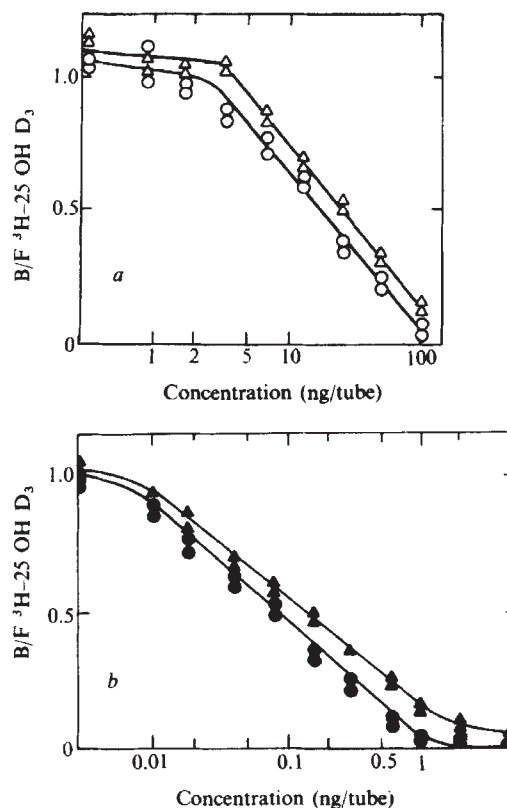


Fig. 2 Displacement of ^3H -25-OH-vitamin D_3 from D-deficient chick plasma with vitamin D analogues. The determination of B/F ratios for comparing binding of D analogues was accomplished with the procedure outlined in Fig. 1 except that chick plasma was used as a source of the specific D binding protein. Symbols as in Fig. 1.

system with chick plasma, increasing amounts of D_2 were ineffective in displacing the ^3H -25-OH- D_3 when compared with D_3 (Fig. 2a). Tests of displacement of the ^3H -25-OH- D_3 by concentrations of D_2 in excess of 100 ng per tube were not possible due to the limited solubility of the fat-soluble vitamin in the aqueous medium of the assay system. 25-OH- D_2 was less efficient than 25-OH- D_3 in displacing the ^3H -25-OH- D_3 from the chick transport protein (Fig. 2b); fifteen times more 25-OH- D_2 than 25-OH- D_3 (w/w) was required to produce 50% displacement. Similar results, that is, marked reduced binding of D_2 or 25-OH- D_2 by blood transport proteins, were found in the plasma of a number of chicks tested.

These findings suggest that resistance to the action of vitamin D_2 in the chick may be due, wholly or in part, to defective binding of D_2 and 25-OH- D_2 by the chick transport proteins resulting in lowered efficiency of the hepatic and/or renal hydroxylations of the vitamin and reduced delivery of the dihydroxy metabolites of vitamin D_2 to target tissues. Further investigation in other species known to be resistant to the action of vitamin D_2 will be important to establish whether similar correlations exist between biological activity and binding to the transport proteins. These results also suggest that selective measurement of vitamin D_3 and 25-OH-vitamin D_3 will be possible using the chick transport proteins, with little interference from D_2 or 25-OH- D_2 . Simultaneous assays⁹ using chick and rat plasma might then prove useful in distinguishing the relative concentrations of D_2 and

25-OH- D_2 (dietary sources) from D_3 and 25-OH- D_3 (sunlight exposure) in man and other species and, thereby, provide insight into the respective importance of environmental and dietary factors in supplying vitamin D requirements in man.

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An Inhibitor of Sterol Biosynthesis present in Cow's Milk

RECENT evidence indicates that sterol biosynthesis from acetate, but not from mevalonate, increases several-fold in the developing rat liver after weaning¹. This increase parallels the postnatal rise in activity of liver 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA reductase), an enzyme which seems to catalyse the rate-limiting key reaction of hepatic sterologenesis both in the adult and in the developing rat^{2,3}. Delayed weaning blocks the normal increase of HMG-CoA reductase which indicates that dietary factors are responsible for the observed changes, and rat milk was shown to contain an inhibitor of HMG-CoA reductase activity².

poration of ¹⁴C-acetate into sterols by liver slices obtained from rats fed milk diet for 3 d was about 50% lower than in the control. Sterol synthesis from ³H-mevalonate, however, was not affected. It should be emphasised that cholesterol content in the standard diet was 0.36 mg g⁻¹ and that defatted cow's milk used in our experiments contained about 8 mg of cholesterol per 100 ml. Since it is well established⁵ that the feedback inhibition of hepatic cholesterol synthesis by exogenous cholesterol requires at least 0.1% of cholesterol in the diet, it seems unlikely that the relatively small amount of cholesterol taken in with the milk would be the cause of the inhibitory effect observed in the rats of groups 2 and 3 (Table 2). Our conclusion is that a dialysable, TCA-soluble inhibitor of sterol synthesis exists in cow's milk. This inhibitor, although effective both *in vivo* and *in vitro*, affects sterol synthesis in a different way when added to the cell-free system

Table 1 Effect of Milk on the Incorporation of ¹⁴C-Acetate and ³H-Mevalonate into Digitonin-precipitable Sterols by 600g Supernatant of Rat Liver

Additions (μl)	Specific radioactivity of sterols			
	³ H d.p.m. mg ⁻¹	% of control	¹⁴ C d.p.m. mg ⁻¹	% of control
None (control)	39,400	100	10,000	100
Cow's milk	10 780	2	—	0
Cow's milk boiled	10 800	2	—	0
Human milk	25 1,900	5	—	0
Human milk boiled	25 2,000	5	—	0
Cow's milk dialysed	25 38,800	100	9,800	100
Supernatant of cow's milk deproteinised with lactic acid, final conc. 5%	25 750	2	—	0
Supernatant of cow's milk deproteinised with trichloroacetic acid, final conc. 5%	25 —	0	—	0
Neutralised lactic acid 5%	50 39,000	100	10,000	100
Neutralised trichloroacetic acid 5%	50 39,000	100	10,000	100

2.5 ml of 600g supernatant of 33% rat liver homogenate in 0.1 M potassium phosphate buffer, pH 7.4, 0.004 M MgCl₂, 0.001 M EDTA and 0.125 M sucrose 'Bucher medium'⁴ was incubated at 37° C for 2 h with 1.0 μCi ¹⁴C-acetate and 0.5 μCi ³H-mevalonate in a total volume of 2.6 ml. Incubation was stopped by adding 15% KOH in methanol and the mixture was saponified at 65° C for 3 h with 4 mg of cholesterol added as carrier. The non-saponifiable lipids were extracted with hexane. The extracts were evaporated and digitonides were prepared according to Sperry and Webb⁶. The digitonides were dissolved in hot methanol and aliquots were taken for both radioactivity and sterol determinations. Sterols were assayed with the Liebermann-Burchardt colour reagent. The endogenous sterol content was 28 mg per 100 ml of supernatant fraction used. ³H and ¹⁴C radioactivity was analysed in a Nuclear Chicago Isocap 300 liquid scintillation counter using the scintillation fluid containing 100 mg POPOP and 4 g PPO per l toluene. The values represent the means of typical experiments run in triplicate.

Here we present evidence that cow's milk, an important component of human diet, exhibits an inhibitory effect on rat liver sterologenesis both *in vitro* and *in vivo*. All experiments were performed on 5 to 6-week-old male Wistar rats. The animals were killed at the same time of day, between 1100 and 1200 h. Defatted cow's milk (fat content 2%, protein content 3.5%) was obtained from state controlled commercial sources, human milk directly from the patients of the Obstetric Clinic. A standard diet for laboratory animals, 'Bacutil'-Poland, called in the table 'solid food', was used, except where stated otherwise. As shown in Table 1, 10 μl cow's milk or 25 μl of human milk added to 2.6 ml of incubation mixture inhibited completely the incorporation of ¹⁴C-acetate and ³H-mevalonate into digitonin-precipitable sterols by liver preparations. The inhibitory activity of cow's or human milk resisted 10 min boiling and was found in the supernatant after protein had been precipitated from cow's milk with 5% lactic acid or 5% trichloroacetic acid (TCA). Overnight dialysis against Bucher medium⁴ completely abolished the inhibitory effect of cow's milk. These facts suggest a low molecular weight and probably non-protein nature for the sterol synthesis inhibitor. It should be noted that, in contrast to our findings, the inhibitory activity of rat milk on HMG-CoA reductase was found to be non-dialysable.

To check whether cow's milk present in the diet could diminish the rate of hepatic sterologenesis, *in vivo* experiments were performed. As shown in Table 2, the incor-

Table 2 Effect of Dietary Cow's Milk on the Incorporation of ¹⁴C-Acetate and ³H-Mevalonate into Digitonin-precipitable Sterols by Rat Liver Slices

Group	Diet	Specific radioactivity of sterols	
		³ H d.p.m. mg ⁻¹	¹⁴ C d.p.m. mg ⁻¹
(1)	Solid food+drinking water (control)	4,470 ± 580	15,400 ± 1,270
(2)	Solid food+milk	4,600 ± 420	7,300 ± 1,290
(3)	Milk only	4,600 ± 310	8,600 ± 1,100

Each group consisted of three rats. The animals were kept for 3 d on the diet indicated. Daily intake of solid food in groups 1 and 2 was about 8.5 g per rat. Daily intake of milk was about 7 ml per rat in group 2 and 12 ml per rat in group 3. 0.5 g portions of liver slices obtained in duplicate from each animal were incubated at 37° for 2 h with 1.0 μCi ¹⁴C-acetate and 0.3 μCi ³H-mevalonate in 1.0 ml of Krebs-Ringer phosphate buffer, pH 7.4 (ref. 7). Incubation was carried out under continuous oxygen flow and was stopped by freezing. Tissue was separated from the medium and saponified at 65° C for 3 h in 5.0 ml of 15% KOH in methanol with 6.25 mg of cholesterol added as carrier. Further experimental details as in legend to Table 1. The endogenous sterol content was 2.2 ± 0.2 mg per g of wet liver. The results of a typical experiment are presented. Values are the means from three animals ± s.d.

than when fed to the intact animal. Whether the inhibitory effect on sterol biosynthesis from acetate and mevalonate is related to the same or different compounds present in milk

remains to be solved. The properties and chemical nature of the milk inhibitor are under investigation.

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DNA Breaks in Rifampin-treated *Salmonella typhimurium* LT2 after Exposure to Nutritionally Complex Media

WE have reported the phenomenon of "minimal medium recovery" of heated *Salmonella typhimurium* LT2 (ref. 1) and how this phenomenon could be explained in terms of DNA damage caused by sequential exposure to heat and nutritionally complex medium². We now show that treatment of *S. typhimurium* with rifampin also results in decreased viability on rich relative to minimal medium and that the DNA from these rifampin-treated bacteria exhibits breaks after exposure to a rich or complex medium.

When exponentially growing cultures of *S. typhimurium* in a glucose-mineral salts medium (M-9)¹ were exposed to 20 µg of rifampin per ml of medium for 15 min, the viable counts on M-9 agar plates remained constant while viable counts on trypticase soy agar, enriched with 0.5% yeast extract (TSY), showed a 99.0–99.9% reduction (Fig. 1).

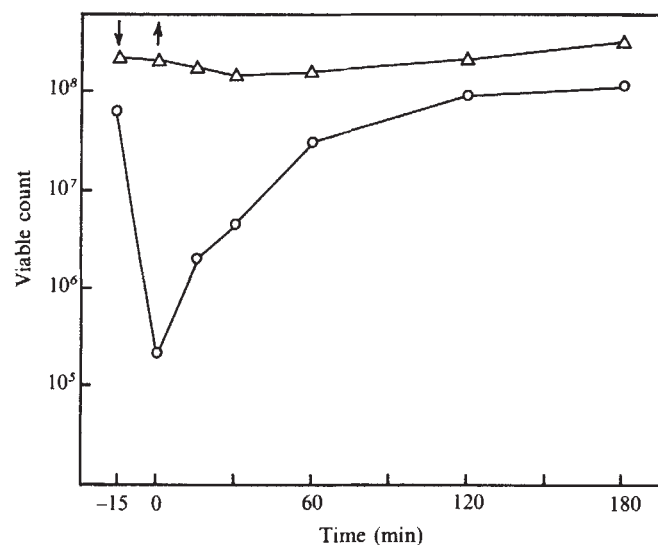


Fig. 1 Behaviour of *S. typhimurium* LT2 during and after incubation in M-9 with rifampin. An exponentially growing culture of *S. typhimurium* in M-9 broth was diluted (1 : 2) with M-9 broth containing 40 µg of rifampin per ml of broth. After 15 min of incubation at 37° C, 10 ml of broth was filtered on a Millipore membrane (HA 0.45 µm; 25 mm) and washed twice with 10 ml of M-9 medium prewarmed to 37° C. The filter was then resuspended in 10 ml of M-9 without rifampin and incubated at 37° C. Viability was assayed on M-9 (△) and TSY (○) plates. ↓, Addition of rifampin; ↑, removal of rifampin.

When the rifampin was removed, the TSY plate counts increased to the same level as before rifampin treatment within 2–3 h. The increase in TSY plate counts indicates repair of the damage produced by rifampin treatment. This observation is similar to the behaviour of heated *S. typhimurium*, although the mechanisms leading to TSY sensitivity may be different for both stresses (heat and rifampin treatments).

Since DNA from heated *S. typhimurium* exhibited breaks after exposure to TSY, we decided to repeat the same experiment with rifampin-treated bacteria. Radioactively labelled cells, grown in M-9 broth to the exponential phase, were treated with rifampin for 15 min and subsequently incubated in TSY for another 15 min. The cells were subjected to alkaline sedimentation analysis after rifampin and again after TSY incubation (Fig. 2a). The sedimentation patterns obtained show that rifampin treatment alone did not alter the weight-average molecular weight of DNA; but, when the rifampin-treated bacteria were exposed to TSY broth, the weight-average molecular weight was reduced ten-fold (Fig. 2b and Table 1). Control experiments demonstrated that exposure to TSY broth alone did not alter the sedimentation pattern of DNA. Likewise, as with heat, cells exposed to rifampin and then TSY lose viability when assayed on M-9 plates (Table 1) and also develop breaks in their DNA.

Table 1 Viability and DNA Weight-Average Molecular Weight of *S. typhimurium* LT2 after Incubation in M-9 with Rifampin followed by Incubation in TSY Broth

Treatment	Viable counts per ml M-9 plates	TSY plates	Weight- average molecular weight *
None	3.0×10^7	1.8×10^7	1.4×10^8
15 min in M-9 + 20 µg ml ⁻¹ RIF	4.0×10^7	4.8×10^5	1.4×10^8
15 min in M-9 + 20 µg ml ⁻¹ RIF followed by 15 min in TSY	2.0×10^6	2.0×10^5	1.2×10^7

RIF, rifampin.

* Computed from Fig. 2.

Figure 1 shows that rifampin-treated *S. typhimurium* recover their resistance to TSY if incubated in M-9 broth without rifampin. Table 2 shows that, in addition to the recovery of resistance to TSY broth, as assayed by viability on M-9 plates, DNA from rifampin-treated bacteria exhibited fewer breaks after incubation in TSY if they were previously incubated in M-9 broth. In other words, repair of rifampin-induced damage can be determined both by the ability of rifampin-treated bacteria to grow on TSY plates and by their resistance to TSY-induced DNA breakage.

Whether or not the reduced survival of rifampin-treated bacteria on TSY plates is a direct consequence of DNA breakage when these bacteria are exposed to TSY, it is interesting that rifampin-treated bacteria suffer DNA breakage when exposed to TSY. Bridges *et al.*^{3,4}, Woodcock and Grigg⁵ and Sedgwick and Bridges⁶ have proposed that DNA breaks after mild thermal treatments occur enzymatically; we concur. Along with Woodcock and Grigg⁵, we propose that endonucleases recognise thermally denatured sections of the DNA molecule. Such enzymes have been generally reported to be present in *Escherichia coli* by Goldmark and Linn⁷ and by Verly *et al.*⁸. In addition, we believe that these endonucleases are activated by TSY. It is then compelling to ask if and how rifampin renders the DNA molecule susceptible to nucleolytic activity.

We would like to advance the following model. It has been proposed⁹ that transcription results in transient disruption of the DNA to DNA base pairing. Recently, it has

also been shown¹⁰ that *E. coli* RNA polymerase can bind to bacteriophage ϕ X174 DNA replicative form and thus pro-

protective form has to be the above mentioned activated RNA polymerase-DNA complex. Whether or not inhibition of RNA synthesis initiation with rifampin yields partially denatured DNA regions remains to be determined. It should

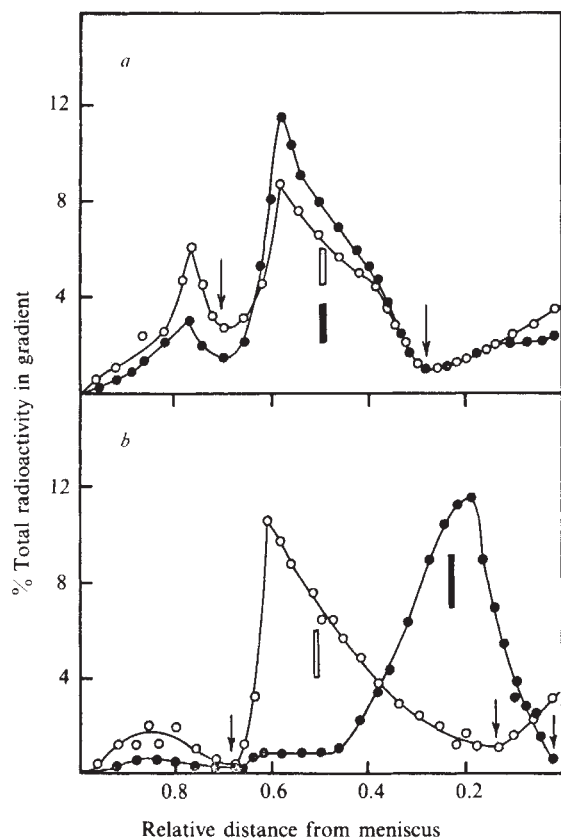


Fig. 2 Alkaline sedimentation of DNA from cells of *S. typhimurium* LT2 cultures incubated in M-9 with 20 μ g rifampin per ml of broth at 37° C for 15 min followed by incubation in TSY for 15 min at 37° C. Cultures of *S. typhimurium* were grown to the exponential phase at 37° C in M-9 broth containing 250 μ g of 2'-deoxyadenosine¹³ and either 15 μ Ci of ³H-thymidine-methyl (6.7 Ci mmol⁻¹, New England Nuclear Corp.) or 5 μ Ci ml⁻¹ of ¹⁴C-thymidine-methyl (51.5 mCi mmol⁻¹, New England Nuclear Corp.) of medium. ³H-labelled cells were exposed to 20 μ g of rifampin per ml of suspension for 15 min (a) and half of the cell suspension was then subjected to alkaline sedimentation analysis² along with half of the ¹⁴C-labelled cells which were untreated and served as internal markers. The remaining half of the ³H-labelled rifampin-treated cells were further incubated in TSY broth at 37° C for 15 min (b) and were then subjected to alkaline sedimentation analysis along with control ¹⁴C-labelled cells. Centrifugation was done in a Spinco SW 50.1 rotor in a Beckman ultracentrifuge (L3-50) at 20° C for 40 min at 40,000 r.p.m. ○, ¹⁴C-DNA; ●, ³H-DNA. Arrows indicate limits within which DNA weight-average molecular weight was calculated². Open and closed bars indicate position of DNA having the calculated weight-average molecular weight for ¹⁴C and ³H-labelled DNA respectively. Sedimentation is right to left. The fast sedimenting fractions in control and some of ³H-labelled cultures are believed to be DNA-membrane complexes¹⁴.

tect specific fragments from endonucleases. Since the addition of rifampin blocks initiation of transcription without affecting RNA chain elongation already in progress¹¹, it is possible that this situation could lead to transient regions of disrupted base pairing not protected by RNA polymerase. These regions would then be susceptible to endonucleases activated by TSY.

Rifampin inhibits RNA synthesis, not by blocking the initial binding of RNA polymerase to DNA^{4,11}, but more likely by inhibiting the transformation of the DNA-enzyme complex to an activated state, where enzyme and DNA change their conformation allowing the enzyme to bind to specific promoter sites on the DNA¹³. Then, if RNA polymerase protects DNA from TSY-activated nucleases, the

Table 2 Effect of Incubation of Rifampin-treated *S. typhimurium* LT2 in M-9 Broth Before Incubation in TSY Broth on Viability and DNA Weight-Average Molecular Weight*

Time in M-9 after rifampin treatment before TSY incubation (min)	Viability on M-9 after TSY incubation (cells ml ⁻¹)	Weight-average molecular weight after TSY incubation †
0	8.0 × 10 ⁵	1.8 × 10 ⁷
30	3.0 × 10 ⁶	2.9 × 10 ⁷
60	8.3 × 10 ⁶	4.2 × 10 ⁷
120	4.0 × 10 ⁶	8.3 × 10 ⁷
240	6.0 × 10 ⁷	1.0 × 10 ⁸
300	7.0 × 10 ⁷	1.5 × 10 ⁸

* Exponentially growing cells, as described in Fig. 2 legend, were exposed to 20 μ g of rifampin per ml of M-9 broth for 15 min and were then washed free of rifampin and incubated in M-9 broth. At different times during M-9 incubation, portions of the cell suspension were exposed to TSY broth for 15 min at 37° C and were then subjected to alkaline sedimentation and viability analysis. Before rifampin treatment there were 6.8 × 10⁷ viable cells per ml as assayed on M-9 plates.

† The DNA weight-average molecular weight before rifampin treatment was 1.5 × 10⁸.

also be mentioned that if rifampin intercalates between the bases of the DNA molecule, then this would also produce local denaturation. However, we do not know of any evidence that rifampin in fact intercalates between the DNA bases.

Indeed, although we have not shown that DNA breakage occurs through endonucleolytic attack, the alternative hypothesis of a physically-induced breakage is hard to visualise. It seems to us that both heat and rifampin cause a change in the cell such that, when exposed to nutritionally rich media, lethal DNA breaks ensue.

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Evidence for Individual Human Peripheral Blood Lymphocytes bearing both B and T Cell Markers

LYMPHOCYTES are composed of two main populations: thymus-derived (T) lymphocytes which are largely responsible for cell-mediated immunity, and bursal-equivalent or bone-marrow derived (B) lymphocytes which are primarily involved in humoral immunity. B cells can be identified by the presence of fluorescence-detectable surface immunoglobulin¹⁻³ and by their ability to bind antigen-antibody complexes or heat-aggregated immunoglobulin through the Fc portion of the immunoglobulin molecule⁴⁻⁷. Human T cells can be identified by their ability to form non-immune rosettes (E rosettes) with sheep red blood cells (SRBC)⁸⁻¹². We report here that when these B and T cell markers were evaluated simultaneously on individual cells, a small subpopulation reproducibly bore both markers. Further experiments seemed to rule out artefact as an explanation.

Lymphocyte separation and fluorescent detection of surface Ig and aggregated immunoglobulin-binding were performed as before^{5,6}. Briefly, peripheral blood lymphocytes (PBL) were isolated from venous blood by Ficoll-Hypaque density flotation followed by passage over nylon fibre columns. The purified PBL were incubated with polystyrene beads and cells adhering to or phagocytosing the beads were discounted. After thorough washing, PBL were incubated with either rhodamine-conjugated anti-human Ig (specific for γ , μ , α , κ , λ) or fluorescein-conjugated heat-aggregated human immunoglobulin, washed again thoroughly, and evaluated by alternate phase contrast and fluorescent microscopy for the percentage of positive cells.

E rosettes were prepared as described before¹², with minor modifications. Purified PBL and neuraminidase-treated SRBC were incubated together at 37° C, pelleted at 200g, and incubated at 4° C for a minimum of 30 min. The pellet was resuspended gently and counted by phase contrast microscopy. Lymphocytes were considered E-rosette-positive if SRBC were adherent. Approximately 90% of E rosettes involved three or more SRBC.

B and T cell markers were evaluated simultaneously on individual cells by first staining the PBL with the appropriate fluorescent reagent, washing thoroughly and then applying

the rosetting technique. Individual cells were then viewed alternately under phase contrast and fluorescent illumination and scored for the presence of one, both or neither marker.

Peripheral blood lymphocytes from nine normal donors (two of the nine subjects were studied twice) were evaluated for percentages of B (surface Ig-bearing, aggregated Ig-binding) and T (E-rosette-forming) cells. Tests for the three markers were performed both separately and simultaneously (surface Ig+E rosettes and aggregated Ig-binding+E rosettes) for each patient on the same day using the same reagents (Table 1). Using the markers separately there was good agreement between markers both for individual donors and the group as a whole. Addition of the percentages for E rosettes and either of the B cell markers accounted for nearly all the lymphocytes (96.7 to 97.7%, range 89.0 to 102.5%). The data agree well with others^{8,13}. Individual donors ranged widely in their populations of B and T cells and the same donor occasionally varied from day to day.

When these B and T cell markers were evaluated simultaneously on individual cells, a small subpopulation (mean 3.6%, range 1.0 to 6.0%) of lymphocytes bearing both markers was observed (Fig. 1). Several observations suggested that the detection of both markers on the same cell was not an artefact. (1) Each individual marker gave equivalent results whether evaluated separately or by the simultaneous technique (that is percentage surface Ig-bearing cells (separate) $\cong$ percentage surface Ig-bearing cells plus double marker cells (simultaneous)). Thus the markers seemed not to interfere with each other, and this suggested that use of the simultaneous technique was valid. (2) Almost all (>95%) double marker cells were heavily rosetted and therefore apparently had at least as high an affinity for SRBC as the usual T cell. (3) It seemed unlikely that the double marker cells were monocytes since they did not adhere to nylon, did not phagocytose and met the morphological criteria of small lymphocytes. Furthermore, monocytes apparently do not bind SRBC under the conditions of the E rosette test^{8,11,12}. (4) An equivalent number of cells bearing both B and T cell markers was observed whether the B cell marker was surface-Ig-bearing or aggregated Ig-binding. These two markers have been shown to be independent⁵⁻⁷. (5) The simultaneous technique using E rosettes formed with non-neuraminidase-treated SRBC (such rosettes

Table 1 Comparative Study of Separate versus Simultaneous B and T Lymphocyte Surface Markers on Normal Human Peripheral Blood Lymphocytes

Subject	Surface Ig	Separate			Totals *	B cell marker	Simultaneous		Both markers	No markers
		Aggregated Ig binding	E rosettes				E rosettes			
Di	39.5†	38.0	55.0	93.0-94.5	Surface Ig	37.0	56.0		5.0	2.0
He	34.5	32.5	64.0	96.5-98.5	Surface Ig	25.0	69.5		3.5	2.0
Co No. 1	28.0	27.5	71.0	98.5-99.0	Surface Ig	23.0	68.0		4.5	5.0
					Aggregated Ig binding	23.0	70.0		3.0	4.0
Co No. 2	25.5	26.0	76.0	101.5-102.0	Surface Ig	20.0	69.0		6.0	5.0
					Aggregated Ig binding	20.0	71.0		4.0	5.0
Br	22.5	20.0	79.0	99.0-101.5	Surface Ig	15.0	75.0		6.0	4.0
Bu	20.0	21.5	81.0	101.0-102.5	Surface Ig	17.0	79.0		2.0	2.0
					Aggregated Ig binding	16.0	77.0		3.0	4.0
Ho	15.0	12.0	77.0	89.0-92.0	Surface Ig	10.0	72.0		5.0	13.0
Ad No. 1	13.7	12.0	84.5	96.5-98.2	Surface Ig	9.0	81.0		5.0	5.0
					Aggregated Ig binding	9.0	82.0		4.0	5.0
Ad No. 2	6.3	6.8	91.0	97.3-97.8	Surface Ig	5.0	82.5		2.0	10.5
Fi	9.0	8.5	84.3	92.5-93.0	Surface Ig	6.0	88.0		2.0	4.0
Ev	7.7	7.0	90.0	97.0-97.7	Surface Ig	7.0	87.5		1.5	4.0
					Aggregated Ig binding	7.0	86.0		1.0	6.0
Range	6.3-39.5	6.8-38.0	55.0-91.0	89.0-102.5		5.0-37.0	56.0-88.0		1.0-6.0	2.0-13.0
Mean	20.2	19.2	77.5	96.7-97.7		15.6	75.8		3.6	5.0

* Totals obtained by addition of percentages for B and T cell markers.

† Numbers represent percentage of cells positive for each marker. Minimum 200 cells counted per experiment.

Table 2 Separate and Simultaneous B and T Lymphocyte Surface Marker Studies on "Clonal" Lymphocyte Populations

Lymphocytes	Surface Ig	Separate Aggregated Ig binding	E rosettes	Totals †	B cell marker	Simultaneous E rosettes	Both markers	No markers
CLL ‡ La	91.5*	90.5	9.0	99.5-100.5	Surface Ig 89.2	9.4	0.2	1.2
					Aggregated Ig binding 88.0	10.0	0.4	2.0
CLL Bi	97.5	96.5	3.0	99.5-100.5	Surface Ig 95.0	4.5	<0.2	0.5
					Aggregated Ig binding 96.3	3.0	<0.3	0.7
CLL St	0.2	0.5	94.5	94.7-95.0	Surface Ig 0.3	93.0	<0.3	6.7
					Aggregated Ig binding 0.3	95.0	<0.3	4.7
Lymphoblastoid line IM-1	97.0	96.5	<0.2	96.5-97.0	Surface Ig 98.0	<0.3	<0.3	2.0
					Aggregated Ig binding 98.5	<0.3	<0.3	1.5

* Numbers represent percentage of cells positive for each marker. Minimum 300 cells counted per experiment.

† Totals obtained by addition of percentages for B and T cell markers.

‡ CLL, chronic lymphocytic leukaemia.

Table 3 Effect of PHA Stimulation of Normal Human Peripheral Blood Lymphocytes on B and T Cell Surface Markers

Condition	Surface Ig	Separate Aggregated Ig binding	E rosettes	Totals †	B cell marker	Simultaneous E rosettes	Both markers	No markers
Pre-culture	13.0*	12.5	81.0	93.5-94.0	Surface Ig 11.0	75.0	2.0	12.0
					Aggregated Ig binding 11.0	77.0	2.0	10.0
PBS	11.0	10.0	82.0	92.0-93.0	Surface Ig 9.0	78.5	1.5	11.0
					Aggregated Ig binding 9.5	78.0	1.0	11.5
PHA ‡	10.5	11.0	83.0	93.5-94.0	Surface Ig 8.5	79.5	2.0	10.0
					Aggregated Ig binding 9.5	81.0	1.5	8.0

* Numbers represent percentage of cells positive for each marker. Minimum 200 cells counted per experiment.

† Totals obtained by addition of percentages for B and T cell markers.

‡ PHA concentration, culture conditions and length of culture were optimised to achieve maximal blastogenic response for the donor.

were more fragile and required longer contact at 4° C between PBL and SRBC) showed the same number of double marker cells. Thus, neuraminidase treatment of SRBC was not responsible for the double marker cells. (6) Double marker cells were not due to anti-SRBC antibodies. Several of the normal subjects (He, Co, Bu) were tested and had no such antibodies by haemagglutination. Furthermore, one patient with infectious mononucleosis and high titre anti-SRBC antibodies showed a percentage of double-marker cells (1.5%) comparable with normal donors. Also, the anti-Ig used in the simultaneous technique would be expected to inhibit SRBC binding by such antibodies. (7) It seemed unlikely that the double marker lymphocytes represented spontaneous antigen binding cells since the latter represent 0.14% or less of the nucleated cells in normal mouse blood¹⁴. (8) The simultaneous technique applied to lymphocytes which had not been passed over nylon fibre showed the same number of double marker cells. Thus lymphocyte contact with nylon was not responsible for the double marker cells.

It was reasoned that if double marker cells were produced by technical factors, then any lymphocyte population studied by the simultaneous technique would show such cells. Conversely, if cells bearing both B and T cell markers were a real subpopulation of PBL, then "clonal" lymphocyte populations should show few or no such double marker cells. One lymphoblastoid cell line and three patients with chronic lymphocytic leukaemia (CLL) were evaluated. The three CLL patients had white blood cell counts $>50,000^{-2}$, and in two cases the cells were of B cell origin and in one case apparently of T cell origin (Table 2). Double marker cells were virtually absent in these populations, thus supporting the view that cells bearing both B and T cell markers are a real subpopulation of PBL.

A markedly increased number of double marker cells (20.0% of PBL) has been encountered in a patient with hypogammaglobulinaemia (manuscript submitted). The meaning of this observation remains to be elucidated.

Attempts to remove (by trypsin digestion) and then regenerate surface Ig in normal PBL were unsuccessful and, therefore, direct evaluation of whether the double marker population represents T cells with the ability to adsorb Ig was not possible. As an alternative approach, normal PBL were evaluated for cells bearing both B and T cell markers both before and after stimulation by phytohaemagglutinin (PHA) in the presence of autologous serum (Table 3). PHA-stimulated PBL gave results equivalent to the controls, that is no increase in double marker cells was noted. Thus, PHA-

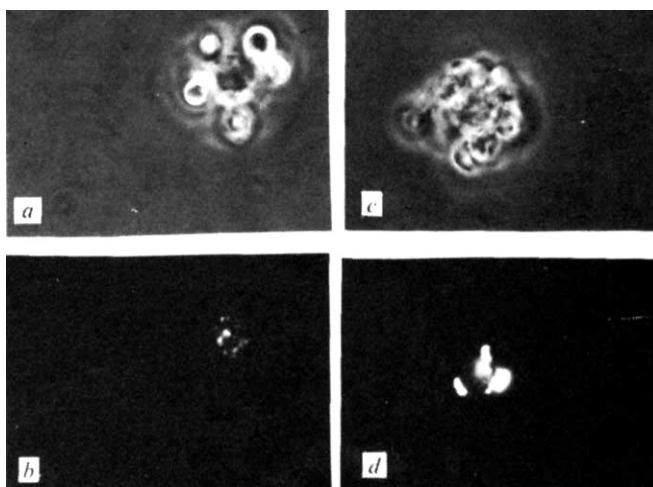


Fig. 1 Demonstration of normal human peripheral blood lymphocytes bearing both B and T cell markers. *a*, Phase contrast illumination of E-rosetted lymphocyte which under fluorescent illumination; *b*, reveals staining with rhodamine-conjugated anti-human Ig. Ig staining is clumpy because of incubation at 37° C during the E-rosette technique. *c*, Phase contrast illumination of E-rosetted lymphocyte which under fluorescent illumination; *d*, reveals binding of fluorescein-conjugated aggregated Ig. ($\times 900$.)

stimulated human PBL did not seem to adsorb Ig. Nevertheless, this does not rule out the possibility that *in vivo* stimulated or antigen-stimulated T cells possess this property.

Lymphocytes bearing neither B nor T cell markers ('null') were also found by the simultaneous technique (mean 5.0%, range 2.0 to 13.0%). This observation must be interpreted cautiously because of the fragility of E rosettes; however, 'null' cells may also represent a real subpopulation, as suggested by studies with the mouse¹⁵.

Other investigators failed to detect lymphocytes bearing both B and T cell markers^{8,9,13}, but our study differed in several respects (methods of lymphocyte purification, specificity of antisera, intensity of fluorescent illumination, methods of preparation of E rosettes) which may account for this discrepancy. On the other hand, Bentwich *et al.*¹¹ reported double marker cells among the PBL of normal donors.

Some murine lymphoma lines have been reported which bear theta antigen, surface Ig and a receptor for Ig^{16,17}. Such tumours could represent neoplastic transformation of a lymphocyte type normally present, and thus provide indirect evidence for the existence of a population bearing both B and T cell markers.

The nature of cells bearing both B and T cell markers remains unclear. Recent reports have presented evidence that some T lymphocytes can adsorb cytophilic antibody¹⁸, and that antigen-activated T cells can bind antigen-antibody complexes¹⁹ and bear easily detectable surface Ig²⁰. If double marker cells prove to be activated T cells, the simultaneous technique should provide a method for identifying and isolating such cells. Alternatively, double marker cells may represent a subpopulation of B lymphocytes which can form E rosettes. Bentwich *et al.* reported that after neuraminidase treatment of PBL, a subpopulation of lymphocytes bearing surface Ig and the Fc receptor will form E rosettes²¹. However, electron microscopy has shown that such binding is different from the binding of SRBC to untreated PBL^{11,21}. Thus, this observation may not be relevant to the data reported here. The third and most interesting possibility is that double marker cells may be able to function as B and (or) T lymphocytes, either as multipotential effector cells, or by further differentiation.

The study reported here provides evidence for the existence of a real subpopulation of lymphocytes bearing both B and T cell markers. Such evidence makes it necessary to reconsider the concept that all lymphocytes belong to one or the other of two distinct classes.

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Autoradiographic Visualisation of Paternal Chromosomes in Mouse Eggs

THE paternal component of the genome has been localised in the nuclear chromatin of early cleaving mouse eggs¹ by autoradiography following the mating of normal females with males in which the spermatogonial cell line was labelled *in vivo* with tritiated thymidine (³H-TdR). The results indicated that in at least the earliest developmental stages of the mouse zygote there is a separation of maternal and paternal chromatin. This extends to mammals the concept of gonamery well known in lower animals². The interpretation of these findings, as well as further exploitation of this experimental system, relies on the distribution of paternal labelled chromosomes at metaphase in early cleavage. An expected separation into twenty labelled and twenty unlabelled chromosomes is most unlikely to occur by chance. Here we present an analysis of the distribution pattern of the labelling in chromosomes.

Mice of the inbred CBA/H-T6/T6 strain were used. Intratesticular injections of ³H-TdR in males, mating with superovulated females, checking of the days of peak production of labelled sperm, collection of zygotes in the oviducts, spreading by air-drying³, preparation and staining of autoradiographs, were performed as described previously¹. The time of collection of the zygotes, calculated from the time of the appearance of the vaginal plug, however, was selected for obtaining eggs close to or at the four cell stage (60 h post-mating). In addition, well-spread metaphases of cleaving eggs were obtained by injecting colchicine directly into the uterine lumen 2 to 4 h before killing the pregnant mice. It must also be pointed out that the intratesticular injection of ³H-TdR results in continuous availability of the tracer for at least 2 d (ref. 4).

Out of 428 well-fixed eggs from 264 females examined, 129 were in metaphase. Of these, forty-one contained twenty labelled chromosomes and eighty-eight had grains in less than twenty chromosomes. Particularly well-spread metaphases allowed arrangement into karyotypes (Fig. 1) in which the paired assortment of homologous chromosomes was established, including the sexual pair and the small translocated T6 marker. The number of labelled chromosomes never exceeded twenty in any metaphases observed, thus giving an autoradiographic visualisation of the classical laws of Van Beneden and Boveri⁵, namely that the number of chromosomes contributed by each parental pronucleus to the diploid zygote is haploid. Labelling of only part of the presumed paternal component, which varied from one to nineteen labelled chromosomes, is probably due to fertilisation by sperm derived from spermatogonia which were exposed to ³H-TdR only during the late part of DNA synthesis. In fact, approximately one-half of the partially labelled metaphases had a labelled chromosome morphologically identifiable as a late-



Fig. 1 Karyotype of a metaphase in a mouse egg at the second division of cleavage. The paternal chromosome in each homologous pair is labelled, the maternal is not, including the XY sexual pair (top left) and the T6 marker (bottom right).

labelling Y chromosome. Moreover, only the Y was labelled in all male metaphases in which only one chromosome contained grains. This is consistent with the late replication of DNA by the Y chromosome before meiosis in male mice⁴. Labelling of autosomes in the partly labelled metaphases varied markedly both in the localisation of grains over different parts of the chromosomes and in the number of labelled chromosomes. The labelling pattern generally corresponded to morphological distinctions previously made on meiotic autosomal bivalents⁴, based on the observation that the late replicating parts of the genome are heterochromatic.

It cannot be excluded that tritium-induced lesions cause morphological anomalies. Even if these may have been missed because of the previous selection of morphologically well-preserved eggs in which extensive exchanges between homologous chromosome pairs had not occurred, the observed labelling pattern cannot have been generated by radiobiological effects.

It must also be emphasised that the relative proximity of labelled or unlabelled chromosomes on spread metaphase preparations does not necessarily reflect their relative position in the living unfixed egg. Indeed, phase contrast microscopic examination of the eggs during the fixation and spreading revealed distortion of the original interchromosomal arrangement.

These results indicate that an expected distribution of

labelled chromosomes on metaphase plates at subsequent mitoses can be observed in eggs fertilised by ³H-TdR-labelled sperm.

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Allelic Contracomplementation Possible Mechanism for Initiating Sympatric Speciation

IN *Drosophila melanogaster*, certain heterozygous combinations of viable alleles of the sex-linked *Abruptex* locus are lethal. This new type of allelic interaction has been called contracomplementation¹. The phenomenon is equivalent or related to negative complementation, but the term contracomplementation is more descriptive in this specific case. The following heterozygous combinations of viable *Abruptex* alleles are found to be lethal: Ax^{9B2}/Ax^{22} , Ax^{9B2}/Ax^{16172} (ref. 2), Ax^{28}/Ax^{71d} (ref. 1), Ax^{71d}/Ax^{9B2} , and Ax^{28}/Ax^{16172} . Interallelic complementation has been supposed to have certain evolutionary significance; it is an intermediate stage in the evolution of heteromultimeric proteins, such as human haemoglobin, from homomultimeric proteins³. Thus, interallelic complementation may be a stage in the progressive evolution of a population. Contracomplementation as an opposite of complementation should accordingly have an 'opposite' evolutionary significance, being an initiation mechanism in splitting one population into two parts, that is an initiation mechanism of sympatric speciation.

Sex-linked genes of different laboratory stocks of *D. melanogaster* which carry contracomplementing alleles of the *Ax*-locus never recombine (except by laboratory manipulations). Supposing that contracomplementing alleles are in the autosomes, stocks carrying such alleles are conceptually different species because they are reproductively isolated from each other. If we presume that a uniform natural population consists of a^+/a^+ individuals and two contracomplementing mutations, a^x and a^y , occur with a certain rate in this population, the frequency of the mutant alleles will increase if both mutations are favoured by selection (a^x/a^x , a^x/a^+ , a^y/a^y , and a^y/a^+ individuals have greater fitness than a^+/a^+ individuals). If, because of contracomplementation, a^x/a^y individuals are lethal or otherwise less fit than other genotypes, the population will virtually split into parts, one containing a^x alleles and the other a^y alleles because genes prohibiting mating between individuals carrying a^x and a^y alleles become beneficial. It is thus suggested that contracomplementation is a possible initiation mechanism of sympatric speciation but the allelic interaction process itself should be studied more profoundly.

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No Mouse PMN Leukocyte Depression after Inoculation with Brain Tissue from Multiple Sclerosis or Spongiform Encephalopathies

RECENT experiments by Carp *et al.*^{1,2} and Licursi *et al.*³ have excited hopes among investigators of multiple sclerosis (MS) and slow virus infections of the central nervous system that an experimental marker for these diseases has finally been found. Tissues from humans with MS or from scrapie mice were inoculated into normal mice, which showed a polymorphonuclear leukocyte (PMN) depression beginning within 1 to 3 d of the inoculation and persisting many months thereafter.

We report here our failure to confirm these results for MS and scrapie or to obtain a PMN depression with the human subacute spongiform virus encephalopathies, kuru and Creutzfeldt-Jakob (C-J) disease.

C57Bl/6J mice were obtained from the Jackson Laboratories, Bar Harbor, Maine. They were distributed into test groups of not more than six mice per cage and held for 2 weeks before experiments were begun. No fighting was observed. No antibiotics were added to their diet of water and mouse chow.

Scrapie mouse brain was used from tenth mouse brain passage of the Compton strain, originally isolated from sheep and previously passed nine times in goats. Normal mouse brain was prepared from healthy adult NIH Swiss-Webster mice. Kuru chimpanzee brain was obtained from chimpanzee A191, inoculated with fourth passage chimpanzee brain material from the Eiru strain. This animal showed the typical clinical and pathological features of kuru, and the inoculum used in the present experiment has transmitted kuru in subsequent chimpanzee passage. C-J chimpanzee brain was obtained from chimpanzee A122, inoculated with first passage chimpanzee brain material from the Woolfe strain. This animal showed the typical clinical and pathological features of C-J disease, but its tissues have not been re-inoculated into primates. Normal chimpanzee brain was obtained from an uninoculated healthy young adult animal. Each of the above brain tissues was ground in a mortar and pestle using sterile sand, diluted to a 10% (w/v) suspension in phosphate-buffered saline, pH 7.4, centrifuged for 20 min at 2,000 r.p.m., and the supernatant fluid stored at -70° C until use. Human MS brain was prepared from autopsy material obtained from patient L. K. who had had a characteristic relapsing illness for 5 yr and had multifocal plaques throughout the white matter of the brain. A coronal section was dissected into grey and white matter, the tissues separately homogenised in an ice-cooled Sorvall blender for a total of 1 min, diluted to a 10% (w/v) suspension in Eagle's Basal Medium, and after centrifugation for 20 min at 2,000 r.p.m. the supernatant fluid was stored at -70° C until use. In addition, Dr Richard Carp provided coded aliquots of a pooled suspension of 10% (w/v) normal human brain and MS human brain. Details of their preparation are described elsewhere¹.

All inoculated mice received 0.03 ml volumes of the dilutions intracerebrally. For leukocyte measurements, blood was drawn into capillary pipettes from which a drop was released for the immediate preparation of slides. 20 µl of the remaining blood was quickly mixed with 10 ml of Isoton in plastic cuvettes to which 3 drops of Zap-Isoton was added, and the white blood cell (WBC) counts measured in a Model B Coulter Counter. The Wright's stained slides were coded and a 200 cell differential count was performed to

obtain the percentage of PMN cells. The code was broken only at the end of each experiment.

Results of the PMN counts in each of three experiments are shown in Table 1.

In experiment one, groups of ten mice were inoculated with each tissue homogenate, and each inoculum group was separately caged. Individual mice in each cage were identified by an ear snip pattern. Mice were bled retro-orbitally without anaesthesia, and each mouse was bled before inoculation and weekly thereafter for 4 weeks.

Instead of a depression there was an elevation of the PMN count in all groups, and the differences between groups were not significant. If only those counts lower than 20% are analysed, the total number of post-inoculation determinations is reduced from 200 to 129, and the average PMN counts to between 12 and 15%—again with no significant differences between the groups. WBC counts were also elevated from pre-inoculation averages between 6,500 and 9,000 cells mm⁻³ to levels from 10,000 to 13,500, with a tendency for entire cages of mice to show comparable elevations in any given week. The mice inoculated with normal mouse brain and those inoculated with scrapie mouse brain were held several more months. All scrapie mice died between 5 and 7 months following inoculation. All normal mice remained well.

Table 1 Percentage of PMN in C57Bl/6J Mouse Blood Before and After Intracerebral Inoculation of Brain Tissue Extracts

Inoculation group	0	1	2	3	4	Post-inoculation average
Experiment 1						
Normal mouse	12	15	12	24	24	19
Scrapie mouse	9	15	17	17	19	17
Normal chimp	8	16	13	21	14	16
Kuru chimp	9	15	14	16	17	16
C-J chimp	10	26	22	19	24	23
Experiment 2						
Uninoculated	10	16	13	11	11	13
MS (white)	10	11	15	—	15	13
MS (grey)	10	11	14	12*	13†	13
MS pool	10	13	8*	13‡	12	11
Normal pool	10	12	16	12*	14	13
Experiment 3						
Uninoculated	14	13	8	9	9	10
Scrapie mouse	14	12	—	13	9	11
MS pool	14	13	11	10	8	10
Normal pool	14	16	8	8	10‡	10

All percentages are based on 200 cell differential counts of Wright's stained blood smears. There are ten mice per experimental point in experiment 1, four mice per point in experiment 2 and five mice per point in experiment 3.

* 3 mice in group.

† 2 mice in group.

‡ 4 mice in group.

In experiment two, sixteen cages containing six mice per cage were handled as follows. One mouse from each cage was bled for control counts on the day of inoculation. The remaining five mice were identified by an ear snip pattern, one was inoculated with each of the four tissue homogenates and the fifth mouse left uninoculated. Four cages of mice were bled and then discarded each week for 4 weeks (each mouse was thus bled only once) giving four mice per experimental point. Mice were bled retro-orbitally without anaesthesia.

From a pre-inoculation average PMN count of 10%, slight average elevations occurred which were not significantly different one from another. The single exception was a depression during the second week in the group of mice inoculated with the pooled human MS brain. This depression was no longer evident in the following 2 weeks, and the combined 4 week values were not significantly

different between groups. WBC counts remained in the 5,000 to 10,000 range throughout the experiment.

In experiment three, sixteen cages containing six mice per cage were handled as in experiment two, except that, following the control bleeding, all mice in each of four cages were inoculated with a given tissue homogenate, and four cages of mice were left uninoculated. Each week for 4 weeks one cage of five mice having received one of the homogenates and one cage of uninoculated mice were bled and discarded. Mice were anaesthetised with ether for 30 s and bled from the exposed axillary vessels.

From a pre-inoculation average of 14%, the PMN counts tended to fall slightly, but in no case was the depression significantly different between the inoculated and uninoculated groups of mice. (One cage of scrapie-inoculated mice had PMN counts between 30 and 75% 2 weeks after inoculation and are omitted from the table.) The combined 4 week values were virtually identical, and not significantly lower than the pre-inoculation average. WBC counts remained in the 4,000 to 9,000 range throughout the experiment.

WBC and PMN counts from the first experiment suggested that a leukocyte stimulus was operating in all groups of mice irrespective of the inoculum given. Cage infections appeared the most likely cause, but sporadic eye infections, anxiety of the unanaesthetised mice, and repeated bleedings of the same mice may also have contributed to the results. Whatever the stimulus, it was uninfluenced by inoculation of scrapie, kuru, or C-J disease-infected brain compared with normal brain. This was equally true when instead of all the data, 'high' PMN counts ($\geq 20\%$) were excluded from analysis. Thus it could be said that if a PMN depressing factor was in fact produced by the infected brain homogenates, it was totally overcome by exogenous leukocyte stimulation.

In the second experiment, by using each mouse only once, randomising the mice within cages, and including uninoculated controls, we were able to avoid the leukocyte stimulus seen in the first experiment. We also included inocula given by Dr R. I. Carp from MS and normal brain tissue that had produced in his laboratory definite PMN depression (MS) and no PMN depression (normal). We found no effect from either these or our own inocula.

A final experiment was then done in which mice were anaesthetised and bled from the axillary vessels, duplicating the technique used by Carp. We also used new aliquots from his MS and normal brain pools. The scrapie material used, as also in our first experiment, was that which we had originally given to Carp for his experiments^{2,3}. Once again, we were unable to distinguish between any of the inoculated groups and the uninoculated group, although there was an overall trend to slightly lowered PMN counts during the 4 week period of study.

We must thus conclude that in spite of efforts to duplicate exactly the protocol of the Staten Island group, using the same strain of mice purchased from the same source, some of the same inocula, and the same methods (including the bleeding technique), we cannot confirm that either scrapie or MS brain tissue inoculated into mice depresses the PMN count, nor have we seen any effect in mice inoculated with brain from kuru or C-J disease.

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Sensitivity of Human Foetal Intestine to Interferon

ACUTE infectious non-bacterial gastroenteritis is a common disease in which recovery usually occurs within 24–48 h of the onset of diarrhoea and vomiting^{1,2}. This short duration suggests that a mechanism of rapid onset, such as interferon, may play a significant role in recovery. The role of interferon in recovery from acute enteric viral infections of man is, however, unknown. Indeed, little is known about the ability of the human intestine to either produce or respond to interferon. We have therefore investigated the role of interferon in viral infections of the human gut. We found that human foetal intestine is quite responsive to the antiviral properties of exogenous interferon, and when so treated exhibits a marked inhibition of the replication of an enteric virus, echovirus type 11.

Previous studies of interferon in intestinal secretions have yielded little information because of the rapid proteolytic enzymatic inactivation of interferon, in spite of the addition of trypsin inhibitors³. We have also found that interferon is inactivated completely when added to human duodenal secretions at a final concentration of 500 U ml⁻¹, even when the secretions first have soybean trypsin inhibitor type II-S added at a concentration of 0.67% or foetal calf serum at a concentration of 16%.

A new technique for maintaining fragments of human foetal small intestine in organ culture for up to 3 weeks⁴ presents a unique opportunity to study the role of interferon in human intestine, free from proteolytic enzymes. Unlike monolayer cell cultures, organ cultures maintain the *in vivo* anatomic relationships as well as certain physiological functions of the intestine⁴. Human foetal intestinal organ cultures were prepared and maintained as previously described⁴. Human interferon was prepared in human leukocytes stimulated by ultraviolet irradiated Sendai virus, using the method of Cantell⁵, and was provided by Drs S. Baron and C. Buckler.

Figure 1 summarises the procedure and results of an experiment in which organ cultures were pretreated for 18 h with exogenous human interferon and then challenged with echovirus 11. In the absence of interferon, echovirus 11 replicated to titres comparable with those reported in intestinal organ cultures⁶. By contrast, cultures pretreated for 18 h with 5 U ml⁻¹ (reference units, see below) or more of interferon failed to support virus replication. Interferon pretreatment with less than 5 U ml⁻¹ permitted virus growth. It is impossible at present to study in this way the replication of aetiological agents of acute infectious non-bacterial gastroenteritis, such as the Norwalk agent², because there is no assay system available except human infectivity².

Having demonstrated the sensitivity of human foetal intestinal organ cultures to interferon, we attempted to determine whether these cultures could be stimulated to synthesise interferon. Several potential inducing agents were used, including polyribonucleosinic polyribocytidylic acid⁷ (poly(I):poly(C), Microbiological Associates, Bethesda, Maryland), echovirus type 11, and the Norwalk agent from a filtrate known to be infectious for man. Several other techniques which augment interferon production were also used; these included interferon priming⁸, diethylaminoethyl (DEAE) dextran^{9,10} (Pharmacia), and treatment after induction with cycloheximide^{11,12} (Sigma). Table 1 summarises

Table 1 Attempts to Stimulate Interferon Production by Human Foetal Intestinal Organ Cultures

Experiment	Priming with human interferon 10 U ml ⁻¹		Inducing agent*		Post-induction augmentation		Interferon assay
	5 h incubation at 36° C	18 h incubation at 36° C	Poly (I) · poly (C) 50 µg ml ⁻¹ + DEAE-dextran 100 µg ml ⁻¹ for 90 min	Echovirus 11, 1,000 TCID ₅₀	Norwalk agent 0.3 ml of 10 µg ml ⁻¹ 1 : 10 dilution	Cycloheximide 10 µg ml ⁻¹ for 3 h	
1	—	—	+	—	—	—	Negative/negative
2	+ †	—	+	—	—	—	Negative/not done
3	—	+	+	—	—	—	Negative/negative
4	—	+	+	—	—	+	Negative/negative
5	—	—	+	—	—	+	Negative/negative
6	—	—	—	+ ‡	—	—	Negative/negative
7	+	—	—	+	—	—	Negative/negative
8	—	—	—	—	+	—	Negative/negative
9	+	—	—	—	+	—	Negative/negative

For priming, interferon was added in 1.25 ml of media to cultures, incubated as indicated, and cultures were then washed three times with fresh media and exposed to inducing agent.

For poly(I)·poly(C) and DEAE-dextran induction, poly(I)·poly(C) of a lot known to induce interferon in human leukocytes and DEAE-dextran were added in 1.25 ml of media to cultures as indicated. Plates were then washed three times, renewed with media, incubated at 36° C for 20 h, and fluids were then collected, as were explants, and frozen at -80° C until assayed for interferon. In experiments 4 and 5, cultures were incubated as indicated with cycloheximide, after removal of poly(I)·poly(C)-dextran, then washed three times, renewed with media, incubated for 20 h, and fluids and explants were then collected.

For virus inoculation, echovirus 11 or Norwalk agent (8FIIa filtrate) was added in 1.5 ml of media to cultures as indicated. Plates were then incubated at 36° C for 20 h, and fluids were collected, as were explants, and frozen at -80° C. Explants from duplicate plates were freeze-thawed three times in 1.5 ml media, and homogenised in a glass tissue homogeniser. Clarified fluid collections and tissue homogenates were ultraviolet-irradiated for 8 min at 2.5 inches from an Atlantic germicidal 282L-20 ultraviolet light bulb, an intensity shown to inactivate completely the infectivity of 10⁶ TCID₅₀ of echovirus 11.

Interferon assay was performed on undiluted and 1 : 5 diluted media and explant homogenate collections, using the VSV cytopathogenicity inhibition assay in human foreskin fibroblast cells.

* Induction was also attempted using 100, 50, 10 and 1 µg ml⁻¹ of poly(I)·poly(C) alone for 1 and 18 h.

† This experiment was also performed using 100 U ml⁻¹ of interferon.

‡ This experiment was also performed using 10⁶ TCID₅₀ echovirus 11.

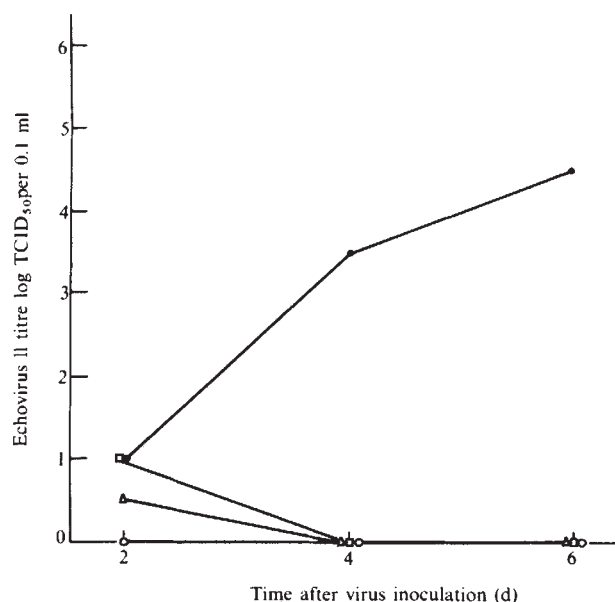


Fig. 1 Effect of exogenous human interferon on echovirus type 11 replication in human foetal small intestinal organ cultures. Organ cultures were prepared from foetal intestine 12–18 weeks old, and maintained for 5 d at 36° C, with the media changed every other day before the start of experiment. Cultures were pretreated with the indicated doses of human interferon in 1.25 ml media for 18 h and then washed three times with fresh media. Echovirus 11, 100 TCID₅₀, was then inoculated per culture dish for 4 h at 36° C; cultures were then washed three times with fresh media, renewed with appropriate interferon-containing media, and incubated. Fluids were collected and cultures refed with appropriate interferon-containing media on days 2, 4, and 6 after virus inoculation. All experiments were run on duplicate plates with pooling of collected fluids. Assays for echovirus in collected fluids were performed in WI-38 cells. ●, Control; ○, 20 U ml⁻¹ interferon; □, 10 U ml⁻¹ interferon; △, 5 U ml⁻¹ interferon.

using the VSV cytopathogenicity inhibition assay¹³. These results were obtained in assays in which known positive control preparations of 5 U ml⁻¹ of human leukocyte interferon could be detected. The standard reference human interferon 6,787 titres 100 U ml⁻¹ by this method and the standard reference human interferon 6,919 titres 5,000 U ml⁻¹ by this method.

Our demonstration of the sensitivity of human foetal intestine to exogenous interferon was made possible by a newly developed intestinal organ culture technique. This technique has obviated in part the experimental difficulties in studying the role of interferon in the gastrointestinal tract posed by proteolytic enzymes in intestinal secretions. Indeed, because of the rapid inactivation of interferon in intestinal secretions, it is doubtful whether intraluminal interferon plays any role in the recovery from viral infections of the gastrointestinal tract. Rather, it is possible that interferon may spread cell-to-cell within the intestinal mucosa, as has been suggested in other tissues¹⁴.

The inability to demonstrate synthesis of interferon by human foetal intestinal organ cultures does not necessarily imply that adult intestine *in vivo* cannot produce interferon. On the other hand, it may be that while the intestine is sensitive to the effects of interferon, cells normally present in adult intestine are unable to produce interferon. In this regard, we have recently demonstrated that one of the most significant pathological features during acute illness with the Norwalk agent of non-bacterial gastroenteritis is a marked round cell infiltration in the lamina propria of the proximal small intestine¹⁵. It is known that lymphocytes possess the ability to synthesise interferon^{16,17}. Conceivably, the round cell infiltrate induced by the Norwalk agent could synthesise local interferon, which in turn could protect the intestine from further viral replication and account for the rapid recovery usually seen in acute infectious non-bacterial gastroenteritis.

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the procedure and results. In no case could we demonstrate interferon synthesis by human foetal intestinal organ cultures

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Hydroxyapatite formed from Coral Skeletal Carbonate by Hydrothermal Exchange

We have prepared porous microstructures, having potential for prosthetic applications, consisting of hydroxyapatite and of whitlockite, by employing exchange reactions at elevated temperatures and pressures. Rather demanding specifications are to be met by biomaterials being considered for tooth or bone replacement. Porous solids have the advantage of allowing circulation of body fluids and of increasing the potential for firm attachment of body tissue^{1,2}. Weber *et al.*³ successfully replicated the porous structure of echinoid skeletal material in epoxy resin and in sodium silicate; and White *et al.*⁴ achieved replication of the coral *Porites* structure with methacrylate, tin, Tichonium, and sintered Al₂O₃. This was the starting point for our attempts to reproduce such structures in the phosphatic material which is a major component of human teeth and bones, hydroxyapatite. Such a material, porous, inorganic and sterile (formed at high temperatures and pressures), should be highly compatible with body tissue, and when used as bone implants may become essentially integral with the bone. Furthermore, composites of such porous hydroxyapatite with other materials including metals⁴ may achieve optimum mechanical and physical properties for a particular application.

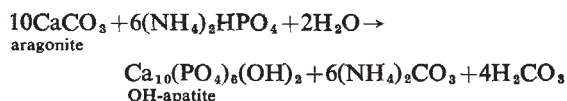
We anticipated that by choice of suitable experimental parameters it would be possible to replace with phosphatic material the porous calcium carbonate skeletal structure of some marine invertebrates. Hydroxyapatite [Ca₁₀(PO₄)₆(OH)₂]

is harder (Mohs hardness=5) than either the aragonite (=3.5–4) or calcite (=3) forms of CaCO₃ and therefore such exchange has the potential to form structures which are stronger than the skeletal material of the original marine organism. It would, of course, have the advantage that it would be chemically and mineralogically 'identical' with normal human hard tissue, and would be stable in contact with body fluids, while the carbonate is not. High pressure and high temperature treatment would furthermore solve problems of sterilisation.

Slices of the massive scleractinian coral, *Porites*, and spines of the asteroid *Acanthaster planci* were used as starting materials to provide the aragonite and calcite polymorphs of CaCO₃, respectively. Hydrothermal techniques⁵ were used, since it was expected that elevated temperatures and pressures would hasten any replacement process. The sections of *Porites* coral, or of the *Acanthaster planci* spine, together with weighed quantities of reactants and water were sealed in a gold tube, heated at specified temperatures and pressures for periods of time varying from 12 h to 1 week, cooled and the products examined.

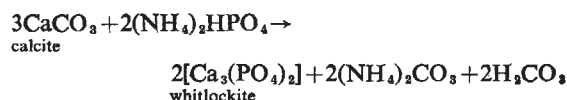
Essentially complete replacement of aragonite by phosphatic material has been achieved under particular conditions. The porous interconnecting structures were preserved, as shown in the scanning electron micrographs of Fig. 1a and b. Figure 1a shows the material which is hydroxyapatite, having replaced the original *Porites* aragonite⁴ and preserved its structure.

Typical experimental conditions for the exchange reaction to produce hydroxyapatite are given in Table 1, along with data on the asteroid material. Experiments resulting in replacement of the aragonite are listed and, for contrast, examples of less favourable conditions. We assume that under favourable conditions the following exchange reaction takes place.



Observations under the polarising microscope are recorded as well; and phase identification was made by X-ray diffraction as well as observations under the microscope.

The *Acanthaster planci* spines were completely converted to whitlockite (β-3CaO.P₂O₅) as shown in Table 1 and Fig. 1b. For the formation of whitlockite, the previous reaction may be modified accordingly:



The microstructure of the whitlockite product is very close to that of the original carbonate skeletal material, shown by Weber *et al.*^{3,4,6,7}.

Some very tentative tensile strength data are given in Table 1, obtained on both the original coral aragonite, and on the hydroxyapatite product. The measurements were made on rectangular bars approximately 0.15 × 0.15 × 1 inch using the 4-point loading method. Whereas the strength measurements were not sufficient in number to be precisely determined, they indicate that the exchange products are generally as strong as or stronger than the original coral, and provide considerable encouragement for extension of the investigation.

The unit cell dimensions of two apatite samples were refined, using the thirteen strongest lines below 2θ=50.5° (Table 2).

There appears to be a real increase in cell volume, ~0.35%, over the reference values for CO₂-free OH-apatite crystallised at higher temperatures. The X-ray peaks are slightly broader than in hydroxyapatite crystallised hydrothermally at higher temperatures. Several effects may account for the cell increase: incorporation of CO₃²⁻, a very small amount of Sr²⁺, and possibly some NH₄⁺, as well as the enlargement which is also observed in hydroxyapatites precipitated at low temperatures.

Semi-quantitative spectrographic analysis data for a typical

Table 1 Hydrothermal Phosphate Exchange of Coral (*Porites*) and Echinoderm Spine Material (*Acanthaster planci*)

Sample number	Reactant	Temperature (° C)	Pressure (pound inch ⁻²)	Time (h)	Result (phase)	Optical or other observation	Microstructure	Strength* g cm ⁻²	Young's modulus g cm ⁻²
Coral									
4h	(NH ₄) ₂ HPO ₄	250	15,000	24	Apatite	Spherulitic birefringence	Open pores replica of coral	3.1 × 10 ⁴	5.2 × 10 ⁶
4g	(NH ₄) ₂ HPO ₄	260	15,000	12	Apatite				
4c	(NH ₄) ₂ HPO ₄ + Ca(OH) ₂	250	15,000	24	Apatite + l.Cc				
5e	(NH ₄) ₂ HPO ₄ + Ca(OH) ₂	260	15,000	48	Apatite				
5d	(NH ₄) ₂ HPO ₄ + Ca(OH) ₂	350	15,000	24	Apatite + Cc	Wavy, spherulitic		2.2 × 10 ⁴	5.0 × 10 ⁶
5c	(NH ₄) ₂ HPO ₄ + Ca(OH) ₂	270	15,000	24	Apatite			3.3 × 10 ⁴	5.8 × 10 ⁶
4i	(NH ₄) ₂ HPO ₄	260	15,000	24	Apatite + v.l. calcite			1.9 × 10 ⁴	4.7 × 10 ⁶
4l	(NH ₄) ₂ HPO ₄	180	15,000	24	Aragonite + apatite	(Partly leaked)	Open pores	2.5 × 10 ⁴	6.0 × 10 ⁶
4j	(NH ₄) ₂ HPO ₄	260	15,000	48	Apatite				
Spine									
2c	(NH ₄) ₂ HPO ₄	260	15,000	24	Whitlockite	Epitaxy partial	Replica of original (partly filled in)		
2d†	(NH ₄) ₂ HPO ₄	260	8,000	24	Whitlockite	(Microstructure more filled in)			
1g†	(NH ₄) ₂ HPO ₄ + Ca(OH) ₂	260	15,000	24	Whitlockite + l. calcite		Replica of original (not as good as 2c)		

* Strength from four-point loading: original aragonite = 2.84×10^4 g cm⁻², Young's mod., = 5.75×10^6 g cm⁻².

† Sea urchin, *Diadema*, rather than *Acanthaster planci*.

apatite derived from coral (No. 4i), after washing in dilute acetic acid, are given in Table 3.

Table 2 Cell Dimensions of Apatite Samples

Sample	a ₀	c ₀	Cell volume
4i	9.435 ± 0.001	6.893 ± 0.001	531.4
5c	9.441 ± 0.002	6.888 ± 0.003	531.7
(reference No. 19)	9.422	6.883	529.2
9, 10*	9.426 ± 0.001	6.884 ± 0.001	529.7
Holly Springs 9, 10*	9.426 ± 0.001	6.884 ± 0.001	529.7

* The two reference measurements made in our laboratory are of synthetic OH-apatite crystallised at 800° C, and of natural OH-apatite (containing impurities) from Holly Springs, Ga. (ref. 9).

Electron microprobe analyses for Ca:P ratio showed approximately the same value as for typical OH-apatites crystallised at higher temperatures, assumed to be stoichiometric.

Infrared absorption spectra taken of typical examples of the exchanged specimens reveal small absorption peaks due to the presence of CO₃²⁻ substituted in the hydroxyapatite structure. The CO₃²⁻ spectra appear to be explained as a composite of peaks at 884, 1,465, and 1,534 cm⁻¹ and of peaks at 864, 1,430, and 1,455 cm⁻¹. This indicates that CO₃²⁻ substitutes both for OH⁻ in the columnar positions, and for tetrahedral PO₄³⁻ (ref. 8). Similar observations concerning infrared absorption spectra have been made by Eysel and Roy^{9,10} in synthetic apatites produced in the same pressure-temperature range from chemical mixtures into which CO₂ has deliberately been introduced. These data suggest that there is a maximum concentration of CO₃²⁻ which may substitute for OH⁻ or PO₄³⁻ under a particular set of conditions.

The full explanation for the phenomenon observed, that hydroxyapatite was formed from aragonite-CaCO₃, while whitlockite was formed from calcite-CaCO₃, has not yet been found, and probably involves complexities of nucleation and crystal growth, as well as phase stability^{11,12}. Indeed, two specimens of coral and spines sealed together in the same tube produced, respectively, hydroxyapatite and whitlockite. Calcite spines of *Acanthaster planci* are magnesium-rich⁶, and magnesium is clearly associated with whitlockite: up to 17 atom % Mg has been found to substitute for Ca in whitlockite¹³,

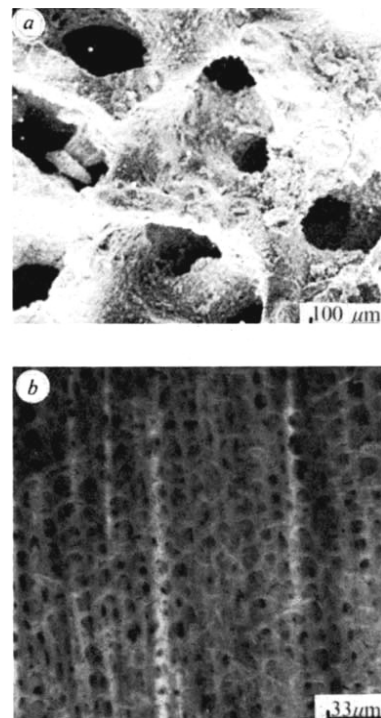


Fig. 1 a, Hydroxyapatite replacement of coral *Porites* structure; b, whitlockite replacement of *Acanthaster planci* spine, originally composed of high-magnesium calcite.

while its solid solution in hydroxyapatite is very limited¹⁷. Naray-Szabo¹⁴ pointed out that Mg²⁺ 'de-stabilises' the apatite lattice; examples are known of whitlockite (rather than hydroxyapatite) formation¹⁵ in dental calculus in which there is positive correlation of whitlockite content with magnesium; and most whitlockite natural mineral deposits contain significant amounts of magnesium.

The major concern of the present work, however, is the exchange process by which the porous coral structure preserved as hydroxyapatite (containing some CO₃) is formed. There are

Table 3 Spectrographic Analysis of a Typical Apatite Derived from Coral

% Oxide	% Oxide
0.2	MgO
0.02	SiO ₂
<0.007	Mn ₂ O ₃
0.007	Fe ₂ O ₃
<0.003	Y ₂ O ₃
1.0	SrO
0.006	La ₂ O ₃
<0.01	CeO ₂
<0.01	Ce ₂ O ₃
0.002	Al ₂ O ₃
0.002	TiO ₂
0.1	NiO
0.05	BaO
0.1	Na ₂ O

Not detected: Cr, Be, Mo, Cu, Ag, Yb, Zn, Cd, Bi, Sb, In, Ge, Ga, Pb, Th, Sc.

certain crystal structural similarities between aragonite and apatite, which obviously favour this morphological preservation¹⁰.

The *Acanthaster* spines consist of single crystal calcite⁷, which could result in ordered epitaxial growth of single crystal whitlockite. While the crystal structure of whitlockite is currently being worked out in detail (A. L. Mackay, reference in ref. 16; and refs 13, 17, 18), the fact that the external morphology is rhombohedral, similar to that of calcite, and its space group (R3c) is similar to that of calcite (R3c), suggests the possibility of such structural control (D. M. Roy and D. Dinger, unpublished).

In conclusion, we have produced porous structures which have great potential for use in human implants, in the material which is the major inorganic component of tooth and bone, and which should be most readily accepted by the body, hydroxyapatite. The second phosphatic material produced, whitlockite, may also have potential for such applications. The hard tissue of the massive scleractinian coral, *Porites*, which was used as the starting material for producing the hydroxyapatite materials, exists in very large quantity, so there is essentially no limit on its availability. Furthermore, the conversion to hydroxyapatite was achieved at elevated temperatures and pressures, insuring more than adequate sterilisation, and preserving the original structure. Such porous materials when used in implants would permit circulation of body fluids, allowing firm attachment of living tissue, to enable regeneration as rapidly as possible.

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Non-electrolyte Probes of Membrane Structure in ADH-treated Toad Urinary Bladder

ALTHOUGH it is well known that antidiuretic hormone (ADH) increases the permeability of amphibian urinary bladders to water and small polar non-electrolytes such as urea and acetamide^{1,2}, there is much uncertainty about the mechanisms involved. To gain further insight into the interaction of ADH with this membrane, we have studied the permeation of lipophilic molecules in the presence and absence of the hormone. It has long been recognised that permeability measurements may offer unique clues about the nature of biological membranes^{3,4}.

Our approach was similar to that used previously to study non-electrolyte permeation across the gall bladder⁵. Urinary bladders from the toad (*Bufo marinus*) were mounted as flat sheets between two lucite chambers. The area of the window between the chambers was 1.3 cm², and each chamber contained 16 ml of saline. The composition of the saline was 104.5 mM NaCl, 2 mM KCl, 1 mM CaCl₂ and 1 mM MgSO₄, buffered at pH 7.4 with 2.125 mM Na₂HPO₄ and 0.375 mM NaH₂PO₄. The saline in each chamber was stirred vigorously with bar magnets, and was gassed with 100% oxygen. The electrical resistance of the urinary bladders ranged from 1,400 to 6,500 ohms cm² with a mean value of 2,600 ± 70 (150) ohms cm². All experiments were carried out at room temperature (22°–24° C).

The permeability of lipophilic non-electrolytes, that is those with an oil/water partition coefficient greater than 1 × 10⁻³, was determined by radioactive tracer techniques. A tracer quantity of the radioactive solute was added to the mucosal solution, and the rate of appearance of the isotope in the serosal solution was determined by withdrawing aliquots of the serosal solution at regular intervals. All radioactive samples were assayed by conventional liquid scintillation counting techniques. Permeability coefficients (Ps) were expressed in cm s⁻¹, and were corrected for unstirred layer effects whenever necessary⁵.

Radioactive ¹⁴C-isotopes were purchased from International Chemical and Nuclear Co., Irvine, California (1,4-butanediol, 1,2-propanediol, antipyrine, caffeine, 1,7-heptanediol, and 1,6-hexanediol), Amersham/Searle Corp., Arlington Heights, Illinois (nicotinamide) and American Radiochemical Corp., Sanford, Florida (n-butyramide and iso-butyramide).

For these non-electrolytes, the unidirectional fluxes reached a steady state within 1 h of addition of the isotope, and remained constant for at least 6 h. ADH ('Pitressin') was added to the serosal solution 2 h after the addition of the isotope to give a final concentration of 130 mU ml⁻¹. Ps of the untreated bladders were calculated from the fluxes during

the hour before the addition of ADH, and the P_s in the presence of ADH were calculated from the fluxes measured in the hour following addition of the hormone.

In the absence of ADH the permeabilities varied from 12 ± 1 (8) $\times 10^{-7}$ cm s^{-1} for nicotinamide to 290 ± 11 (6) $\times 10^{-7}$ cm s^{-1} for caffeine. As anticipated, there was a linear relationship between the permeability coefficients of the lipophilic solutes and their olive oil/water partition coefficients: the slope of the line was 1, and the regression coefficient was 0.86. This indicates that the membrane, or membranes, controlling permeation across this epithelium are as hydrophobic as olive oil, and that the selectivity for both permeability and bulk phase partition coefficients is governed by the same basic intramolecular forces⁴.

Addition of ADH caused a statistically significant increase in the permeation of all solutes across the bladder. The P_s for nicotinamide and caffeine increased to 23 ± 2 (6) $\times 10^{-7}$ and 377 ± 0 (2) $\times 10^{-7}$ cm s^{-1} respectively. The P_s for all nine non-electrolytes in the presence of ADH are plotted against those in the absence of ADH in Fig. 1. This shows

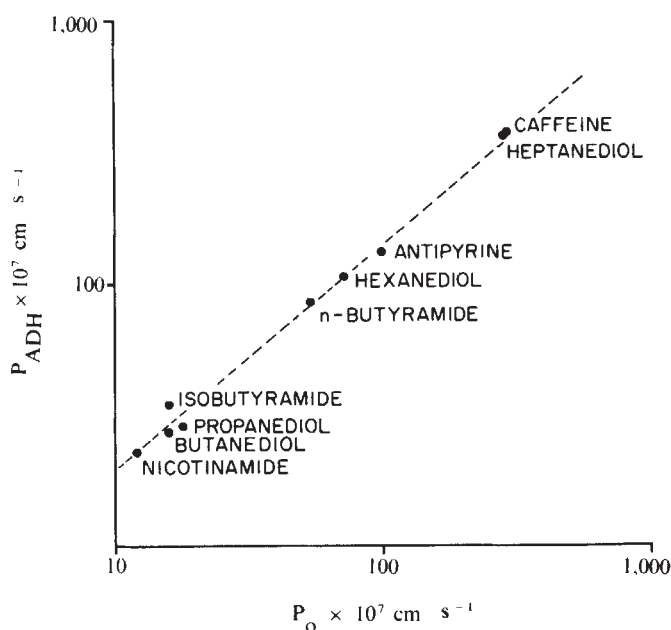


Fig. 1 The effect of ADH on the permeation of lipophilic solutes across the toad urinary bladder. Non-electrolyte permeability coefficients (cm s^{-1}) obtained in the presence of ADH (on the ordinate) are plotted against the coefficients obtained in the absence of the hormone (on the abscissa).

that there is a linear relation between the permeabilities in the absence and presence of hormone; the slope of the line was 1.3, and the regression coefficient was 0.99. This leads to the conclusion that the hormone produces a generalised, but unselective, increase in the permeability of all the lipophilic compounds, that is the permeability of all the compounds increased by roughly the same factor, and there is no apparent change in the selectivity. (Similar changes in permeability were produced by the addition of ADH to the mucosal solution, but only in the presence of 0.5–1 mM LaCl_3 . This suggests that the surface charge on the membrane surface is involved in the action of the hormone.)

The effect of ADH on the permeability of the toad bladder to lipophilic solutes is much smaller than that obtained with hydrophilic solutes. For example, the permeabilities of urea, acetamide and water increased from 14 ± 1 (16) $\times 10^{-7}$, 16 ± 1 (44) $\times 10^{-7}$, and $1,300 \pm 80$ (56) $\times 10^{-7}$ cm s^{-1} to 49 ± 8 (2) $\times 10^{-7}$, 72 ± 16 (14) $\times 10^{-7}$ and greater than $10,000 \times 10^{-7}$ (8) cm s^{-1} respectively.

Many biological membranes discriminate between branched and straight chain isomers⁴. Branched chain solutes are usually much less permeable than the straight chain isomers, and this has been attributed to the highly ordered configura-

tion of the lipids in the cell membrane. In the toad bladder we find that in the absence of ADH the permeability of iso-butyramide is 16 ± 1 (20) $\times 10^{-7}$ cm s^{-1} , whereas the permeability of n-butyramide is 54 ± 3 (20) $\times 10^{-7}$ cm s^{-1} . This difference in permeability is strikingly similar to that for the permeation of the amides across red cell membranes⁶ and gall bladder epithelium (unpublished observations). Thus it may be concluded that the lipids exist in a highly ordered configuration in the epithelial membranes of the toad urinary bladder.

The permeability of both isomers increased when ADH was added, but the increase obtained with iso-butyramide was much more dramatic than with the straight chain isomer. The permeabilities of iso-butyramide and n-butyramide increased to 35 ± 3 (8) $\times 10^{-7}$ and to 68 ± 7 (8) $\times 10^{-7}$ cm s^{-1} respectively. In paired experiments on tissues from the same animal, the ratio of the iso-butyramide to n-butyramide permeabilities increased from 0.30 to 0.51 when the membrane was treated with ADH. These results strongly suggest that the branched molecule encounters less steric hindrance in permeating across ADH treated membranes than in control membranes. This leads to the conclusion that ADH increases the permeability of the toad urinary bladder epithelium by increasing the fluidity of the cell membranes.

In biological and artificial membranes it has been reported previously that increases in membrane fluidity are associated with positive increments in non-electrolyte permeation^{7–10}. Furthermore, recent studies of liposomes showed that when the hydrocarbon tails of the membrane lipids passed from a fluid into a crystalline state, there was a decrease in the partition of solutes into the membrane^{11–14}. This suggests that ADH effects in the toad bladder epithelium result from an increase in the partition of solutes into the plasma membranes, but changes in solute mobility cannot be excluded.

It is now recognised that the partition of solutes into biological and artificial membranes probably varies with position in the membrane; lipophilic solutes are expected to be partitioned mainly into the hydrocarbon core of the membrane, whereas hydrophilic solutes are probably located nearer the polar head groups of the lipids¹⁵. Consequently, changes in fluidity of a membrane could produce quantitatively different effects on lipophilic and hydrophilic solutes. If, for example, ADH caused a relatively larger increase in the fluidity at the periphery than in the core of the bladder membranes, there should be a greater increase in the permeation of hydrophilic solutes than lipophilic solutes. Such an effect may, at least partially, account for the quantitative difference between the effect of the hormone on small polar solutes and lipophilic solutes. This hypothesis could be tested by measuring the effect of the hormone on the partition of solutes into isolated plasma membranes of the toad bladder.

It is still not certain whether or not ADH acts on the apical cell membrane of the toad bladder epithelium directly, or through some intracellular mediator. Evidence for a direct effect on the apical membrane, at least for non-electrolyte permeability, is that (i) vasopressin increases the osmotic water permeability of artificial bilayer membranes, whereas cyclic AMP does not^{16–17}, and (ii) ADH in the mucosal solution increases the permeability of the toad bladder when the surface charge on the outer surface of the apical membrane is screened by polyvalent cations.

In conclusion, we have shown the ADH increases the permeability of the toad urinary bladder to lipophilic solutes, in addition to the effect on small polar solutes and water. This increase in permeability is associated with an increase in the fluidity of the membrane, or membranes, controlling permeation across the epithelium. Evidence is cited to suggest that the change in configuration of membrane lipids acts to increase the partition of solutes into the membrane, and that quantitatively different effects are expected for lipophilic and hydrophilic solutes. These experiments emphasize that a

direct interaction of the ADH with the membranes of the toad urinary bladder may account for at least some effects of this hormone. Further clues will undoubtedly come from experiments designed to evaluate the effect of ADH on permeability of the apical membrane of the epithelium.

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Melatonin stimulates Growth of Brown Adipose Tissue

In small mammals brown adipose tissue (BAT) is an important site of non-shivering heat production^{1,2}. Its mass increases during cold adaptation together with the ability to produce heat (ref. 3 and G. H., unpublished), thus improving cold tolerance⁴. In the golden hamster, Hoffman *et al.*⁵ also found an increase of BAT weight following exposure to short photoperiods. The pineal hormone melatonin has been shown to affect photoperiodic responses (ref. 6, and K. H. and G. H., unpublished), and therefore an action of melatonin on development of BAT seemed likely. In this study, the effect of short photoperiods and of melatonin on the amount of BAT was investigated in the Djungarian hamster *Phodopus sungorus*. This species shows a marked annual cycle of testis size and activity, body weight, and coat colour when kept under natural light conditions⁷, and all of these functions can be influenced by manipulation of the photoperiod (ref. 6, and K. H. and G. H., unpublished). The species does not hibernate, but may show daily torpor in winter, while the gonads are regressed even at room temperature⁷.

Fifty adult male hamsters (average body weight 42 g) that had been kept under natural light conditions and temperatures of $20 \pm 3^\circ \text{C}$ were exposed to either long (16 h light d^{-1}) or short (8 h light d^{-1}) artificial photoperiods at $20 \pm 1^\circ \text{C}$ starting in July. The animals were housed singly. Nine hamsters in either condition were implanted with tablets of one part melatonin in ten parts beeswax (average weight of

implants 34 mg) subcutaneously six times at weekly intervals. Each tablet thus contained about 3.1 mg of melatonin but control experiments showed that only a small fraction of this was absorbed⁸. Control animals in each light condition were either left untreated (seven hamsters each), or received implants of beeswax only (nine hamsters each). After 64 d under artificial light conditions, the hamsters were killed, weighed, and stored in a deep freeze until dissection. Drying was prevented by wrapping each animal in a plastic bag. Body weight was again checked before removal of BAT and no decrease resulting from storage was found. BAT was carefully dissected from the interscapular, subscapular, dorsal, cervical, suprasternal, subvertebral and other intrathoracic sites and was weighed immediately after dissection. No BAT was taken from the perirenal region.

The weight of BAT thus determined is given in Fig. 1 for all hamsters. To eliminate the effect of differences in body weight, weight of BAT was calculated as a percentage of body weight. Control hamsters kept the 8 h photoperiod contained nearly 50% more BAT than those kept in the 16 h photoperiod ($P < 0.001$; *t* test). Hamsters that had received melatonin had the highest amount of BAT; on the average it was more than 5% of body weight. In both photoperiods, BAT mass of hamsters treated with melatonin differed significantly from that of the respective controls ($P < 0.001$). No difference in BAT content was found in the two groups receiving melatonin but kept in different photoperiods.

The finding that the controls contained significantly higher amounts of BAT after exposure to short photoperiods confirms results obtained in the golden hamster⁵, and shows that seasonal increase in BAT mass in winter is not simply a consequence of cold exposure, but can also result from the short photoperiod at that season.

The drastic increase of BAT mass in hamsters treated with melatonin indicates that the pineal may be involved in its regulation. The pineal has been shown to participate in the regulation of seasonal cycles by light^{8,9}. In mammals, melatonin is exclusively produced in the pineal, and the activity of hydroxyindole-O-methyltransferase, the enzyme responsible for the last step in melatonin formation, has been reported to be light dependent, showing high activity in darkness and low activity in light⁸. Our data demonstrate a drastic effect of melatonin on growth of BAT, and it seems reasonable to assume that the rise in BAT content in hamsters kept in short photoperiods might be mediated by increased production of melatonin in the pineal in these conditions. As no difference in BAT was found between the two photoperiods in the hamsters treated with melatonin, and as the amount was nearly twice that found in the controls in short photoperiods, one might speculate that the amount of melatonin effective in our experiments already instigated the maximal possible growth of BAT, and that therefore no additive effects of photoperiod could be observed. This assumption is supported by the fact that the amount of BAT found in our experiments after melatonin treatment is higher than nearly all previously reported values¹.

The pathway by which melatonin acts on the growth of BAT is unknown. Melatonin has been claimed to be the antgonadotrophic hormone of the pineal⁸ but contradictory results have been obtained after its administration^{9,10}. In *Phodopus*, extraneously administered melatonin delayed the accelerating effect of long photoperiods on testis development, increase of body weight, and change into summer coat in midwinter, but did not completely suppress such development⁶. In summer, melatonin suppressed or greatly diminished the effect of short photoperiods which in untreated controls caused testicular involution, decrease of body weight, and change into winter coat (K. H. and G. H., unpublished). In the experiments reported here, however, melatonin caused enlargement of BAT independent of photoperiod. Thus, its action does not parallel that on the

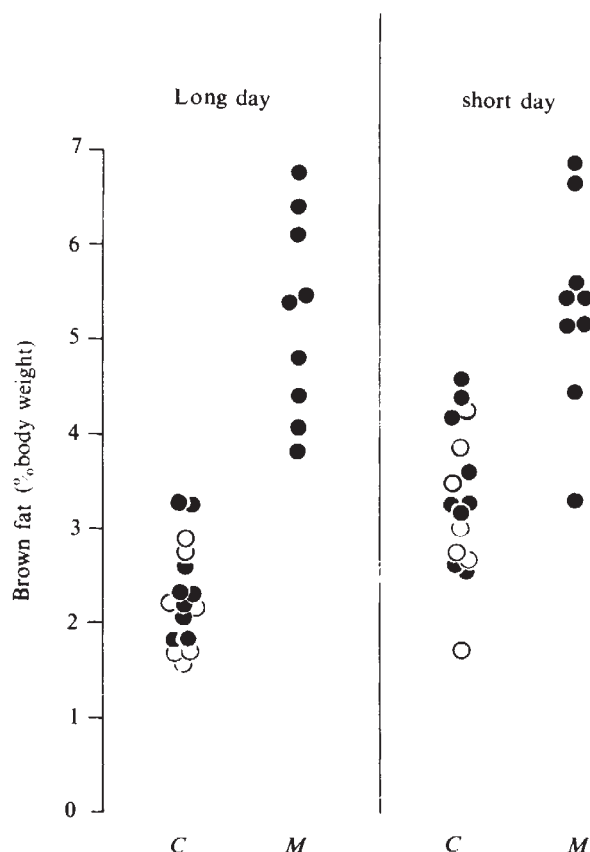


Fig. 1 Weight of brown adipose tissue (BAT) as percentage of body weight in the Djungarian hamster *Phodopus sungorus*. Each point represents BAT weight of a single hamster. M, Hamsters treated with melatonin; C, controls without any treatment (○) and implanted with plain beeswax (●). Mean $\pm$ s.e.: long day, C, 2.30 ± 0.13 ; M, 5.24 ± 0.35 ; short day, C, 3.34 ± 0.20 ; M, 5.33 ± 0.36 . Long day, 16 h light; short day, 8 h light.

other functions showing seasonal variations, and must be brought about by different pathways.

The physiological mechanism controlling development of BAT during cold adaptation is not resolved. There are indications that thyroxine and noradrenaline are involved in stimulation of BAT growth because, in rats, an increase in BAT mass was observed after repeated injections of thyroxine and/or noradrenaline⁴. Blocking the thyroid activity in mice by feeding them with propylthiouracil caused a reduction in the amount of BAT (G. H., unpublished). In young rats, growth of BAT was stimulated by injections of cortisone¹¹. Little is known about the effects of melatonin on the adrenal cortex, but it has been reported to inhibit thyroid function, though the results are far from uniform^{8,12}. As melatonin stimulated BAT growth in our experiments it does not seem to act by way of the thyroid. A direct effect on BAT growth or a completely different pathway seems more likely.

The action of melatonin on growth of BAT described here is, to our knowledge, the first evidence that the pineal may be involved in thermoregulatory adjustments in mammals.

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Transmission of Mammalian Piroplasm by an Argasid Tick

It is the accepted view that parasites belonging to the sub-order Piroplasmidae Wenyon, 1926, are exclusively transmitted by "hard" ticks belonging to the family Ixodidae Murray^{1,2}. Most piroplasms of domestic animals have more than one Ixodid vector. In the laboratory, small mammal piroplasms belonging to the genera *Babesia* Starcovici, 1893, and *Nuttallia* Franca, 1910, are used as models for the diseases caused by piroplasms of domestic animals. This research, however, suffers from the drawback that the parasites cannot be biologically transmitted, except in two cases. Thus, *Babesia rodhaini* Van den Bergh, Vincke, Chardome and Van den Bulcke, 1950, a rodent piroplasm very commonly used in laboratory investigations, has no known vector; nor has *Babesia hyalomysci* Bafort, Timperman and Molineux, 1970. The two small mammal piroplasms of which the vectors are known are *Nuttallia microti* Coles, 1914, and *Nuttallia danii* Tsur, Hadani and Pipano, 1960 (and the probably identical *N. tadjikistanica* (Krylov and Zanina, 1963), Krylov, 1964). The vectors of these piroplasms are *Ixodes trianguliceps*³ Birula, 1895, for the former, and three species of *Hyalomma* and two species of *Rhipicephalus*⁴ for the latter. All of these vectors belong to the Ixodidae.

Nuttallia meri Gunders, 1971, was isolated from the fat sand rat *Psammomys obesus* Cretzschmar, 1828, from the lower Jordan Valley⁵ and, though having a narrow range of infectivity for laboratory animals, is considered a potential model for the study of piroplasmosis of economic importance. The search for possible tick vectors in nature led to the discovery, within *Psammomys* burrows, of the "soft" tick *Ornithodoros erraticus* (Lucas, 1849) (small race) which had not previously been reported from Israel⁶. No other ticks were found in this biotope.

We have reported elsewhere⁷ failure to transmit *N. meri* through clean laboratory-raised *Hyalomma excavatum* Koch, 1844, *Rhipicephalus sanguineus* (Latreille, 1806) and *Ornithodoros tholozani* (Labulbene and Megnin, 1882), but successful infection of clean laboratory-bred, splenectomised, *Psammomys* (HUPO strain) followed intraperitoneal injection of triturated field-collected *O. erraticus*. Further, one laboratory-bred *Psammomys* became infected with *N. meri* after eleven adult ticks were fed on it. The ticks had been collected in the field 2 d earlier. Although there had been a lapse of only 2 d between field collection and biting by the ticks, it was clear that this was true transmission, as the ticks had to be starved to be ready to feed.

The purpose of this communication is to report three further instances of transmission of *N. meri* to *Psammomys* by the bite of *O. erraticus* up to 81 d after field collection of the ticks. All three sand rats were of the Hebrew University strain of *Psammomys obesus*, were laboratory bred and were splenectomised before having ticks fed on them. Four nymphs and three imago *O. erraticus* that had been collected in the field 39 d earlier were fed on the first *Psammomys*. Parasitaemia became apparent on day 17. Eight adult *O. erraticus* and one nymph were fed on the second sand rat 81 d after their collection. The sand rat

became manifestly infected on day 17. A pool of starved ticks were fed on the third *Psammomys*. The ticks included some that had infected the first *Psammomys* on day 39 after collection and others that had been found to be uninfected. Again, transmission of *N. meri* occurred and was observed on day 12, indicating that a batch of ticks may infect more than once and that the parasite persists in the ticks, even after a blood meal, for at least 81 d.

The four instances of transmission, by bite, of *N. meri*, through field-collected *O. erraticus* clearly establish this tick as a natural vector of this parasite. This is the first proven example of transmission of a mammalian piroplasm by an Argasid ("soft") tick. The reports by Rastegaïeff⁸ and Bitukov⁹ suggesting that *Theileria* (= *Gonderia*) *ovis* (Rodhain, 1916) Lestoquard, 1929, was transmitted through *O. lahorensis* Neumann, 1908, are considered doubtful by Neitz (ref. 1 and personal communication) because of the excessively short incubation periods following tick bite (3 and 8 d, respectively) and because the sheep and goats had not been shown to be free of naturally acquired infection before their use as experimental animals.

The demonstration that an Argasid tick, *Ornithodoros erraticus*, could transmit, by bite, a mammalian piroplasm (*N. meri*) suggests that, for those small mammal piroplasms for which no natural vector is as yet known, a search among the soft ticks may be rewarding.

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Carnosine in Nucleated Erythrocytes

In the course of studies of the metabolism of intracerebrally administered L-U-¹⁴C-histidine in brains of mice and frogs (*Rana pipiens*), analyses were also performed on blood samples from the injected animals. The mice received 1.5×10^6 c.p.m. of the labelled histidine, and 1 h later samples of blood were taken by means of orbital puncture. Frogs were injected with 2.9×10^6 c.p.m. and blood was obtained after 1 h by cardiac puncture. The samples were deproteinised in 5 volumes of 0.4 N perchloric acid and processed in the manner described previously for brain and tumour tissues¹. Aliquots of neutralised extracts were electrophoresed on Whatman No. 3 paper at pH 10.0 together with known standards of carnosine, homocarnosine, histidine, imidazoleacetic acid, N-acetylhistidine, and histamine; and stained with Fast Red B in the manner described previously¹.

Scanning the electropherograms showed only a few very minor radioactive peaks, which were not investigated further, but a marked enhancement of the size of the carnosine marker spot was noted in the extracts of frog blood. Electrophoresis without added markers showed a strongly diazo-positive material to be present in the carnosine position in

extracts of frog blood, and subsequent experiments showed it to be largely located in the erythrocytes. Electropherograms of mouse blood were negative.

When neutralised perchloric acid extracts of frog erythrocytes were subjected to two-dimensional thin-layer chromatography (TLC) (ref. 2), the major diazo-positive substance migrated to a position corresponding to that of carnosine (Fig. 1). In addition, there was a small amount of material corresponding in position to N-acetylhistidine on

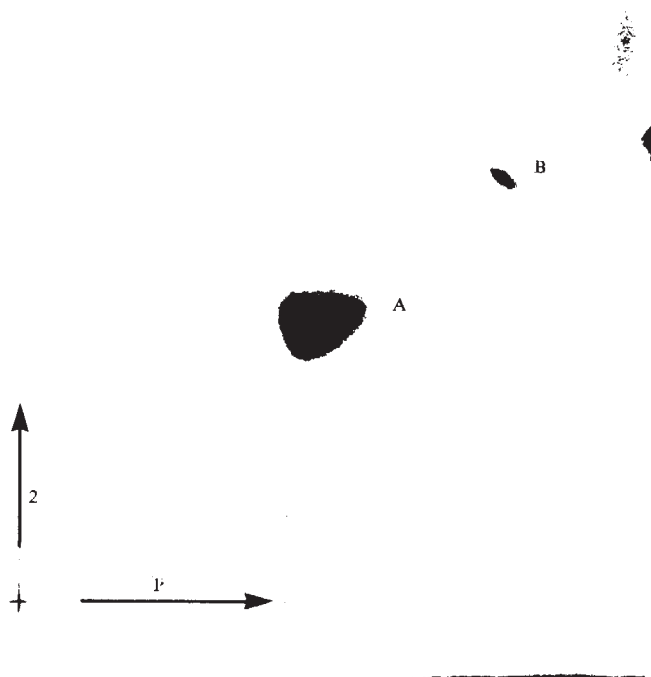


Fig. 1 Two-dimensional TLC on silica gel² of extract corresponding to 20 μ l of packed frog erythrocytes. The chromatogram was run in the first dimension (1) in ethanol: ammonia (28%): water (70:5:25) and in the second dimension (2) in chloroform: methanol: ammonia (28%): water (30:60:5:15). Spot A occurs in a position characteristic for carnosine, and spot B for N-acetylhistidine².

the chromatograms. We have shown previously that, in the frog, intracerebrally injected histidine is converted extensively into N-acetylhistidine¹. When the frog erythrocyte extracts were chromatographed with histidine, carnosine, homocarnosine and N-acetylhistidine (Fig. 2), it became apparent that the unknown material was probably carnosine. Material containing the compound was then isolated from one-dimensional TLC plates³ and a portion was hydrolysed at 110° C in 6 N HCl for 20 h as shown by two-dimensional TLC (ref. 2) of equal aliquots of hydrolysed and unhydrolysed samples. Disappearance of the original material and liberation of β -alanine and histidine were noted on hydrolysis (ref. 2).

Analysis of the above samples by column chromatography on a Beckman amino acid analyser showed there to be 38 nmol of histidine and no β -alanine in unhydrolysed extract corresponding to 1 ml of packed erythrocytes, while after hydrolysis 116 nmol of histidine and 92 nmol of β -alanine were found. Thus, histidine and β -alanine were liberated in approximately isomolar amounts on hydrolysis, confirming the identity of the diazo-positive material with carnosine.

Employing the above techniques, carnosine has been detected in erythrocytes of White Leghorn chickens, but in smaller quantities than in the frog. To date we have not

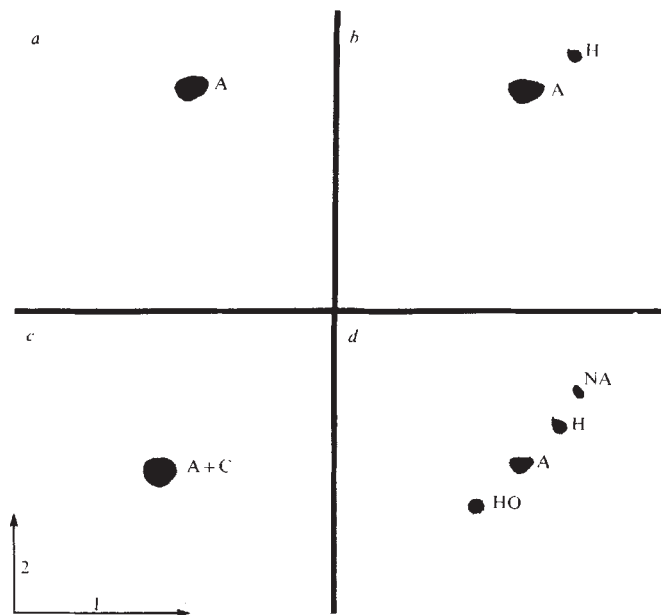


Fig. 2 Two-dimensional TLC of equal amounts of isolated spot A with and without markers run in the same manner as in Fig. 1. Chromatograph *a*, spot A alone; *b*, histidine added; *c*, carnosine (c) added; *c*, together with homocarnosine (HO), histidine (H), and N-acetylhistidine (NA). Solvent system same as for chromatograph of Fig. 1.

been able to detect carnosine in concentrated extracts of mouse erythrocytes.

To our knowledge, carnosine has not been detected previously in erythrocytes of any species. Our results here show the presence of this dipeptide in relatively large amounts in frog erythrocytes and in smaller amounts in chicken erythrocytes, although it was undetectable in mouse red blood cells. Meaningful suggestions about the source or function of carnosine in nucleated erythrocytes cannot be made until much further work is accomplished. Although the presence of carnosine in muscle has been known for over 70 years³, its function is still largely shrouded in mystery.

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Association of Methionine Requirement with Methyl Mercury Resistant Mutants of Yeast

It has been known for several years that strains resistant to mercury can be obtained in several bacterial species¹⁻⁴. Soon after the correlation between resistance to antibiotics and to mercury was recognised^{2,5}, it was established that genetic

elements conferring resistance to antibiotics, mercury and other heavy metals in *Escherichia coli* and *Salmonella typhimurium*⁶ and *Staphylococcus aureus*^{7,8} reside on extra-chromosomal resistance transfer factors or plasmids. Among fungi, mercury resistant strains of *Botrytis cinerea*⁹, *Penicillium notatum*, *Sclerotinia fructicola*, *Stemphylium sarcinaeforme*¹⁰, and *Saccharomyces cerevisiae*^{11,12} have been reported. In most cases^{1,3,9-12}, this was accomplished by 'training' the normal strains for growth on media supplemented with successively increasing concentrations of mercury compounds, and in some cases¹⁰ the resistance was lost when subcultured on mercury-free media. It is noteworthy that in none of the mercury-adapted strains of fungi has the genetic basis of resistance been determined. In this report we describe a method of isolation and characterisation of methyl mercury resistant mutants of *S. cerevisiae*. This study was undertaken with the view that the examination of physiological changes associated with genetically defined resistant mutants will be useful in studying the mechanisms of cellular detoxification of organic mercurials.

We used the following simple technique as the most efficient means of isolating spontaneous and induced methyl mercury resistant mutants. Fresh stationary phase cultures of haploid strains in YPD medium (1% Bacto-yeast extract, 2% Bacto-peptone, 2% dextrose) were washed and treated with 3% ethyl methanesulphonate for 1 h at 30° C. From 5×10^6 to 1×10^7 treated cells were spread on each dish of minimal agar medium (0.67% Bacto-yeast nitrogen base without amino acids, 2% dextrose and 1.5% agar) supplemented only for the nutritional requirement of the original strains. Sterile paper strips or disks were placed in the middle of the plates on which 30 to 60 nmol (100 to 200 μ l of a 3×10^{-4} M solution) of methyl mercury chloride (K and K Laboratories, Plainview, New York) was dispensed. The cells were thus exposed to a continuous concentration gradient of methyl mercury. The plates were incubated at 30° C for 7-10 d. Cells could grow into a confluent lawn at the edge of the plate where the methyl mercury concentration was low and not inhibitory, but they could not grow near the impregnated paper. Occasionally a few colonies appeared in a region where the methyl mercury concentration was inhibitory to most cells. These colonies were isolated as presumptive resistant mutants and tested further. We have also isolated spontaneous and ultraviolet light-induced mutants by this method.

When colonies selected for methyl mercury resistance were analysed, most of them would not grow on minimal medium with or without methyl mercury and with the supplements necessary to satisfy the nutritional requirement of the parent strain. Further tests revealed that these mutants had become auxotrophic for methionine. It should be noted that the selection medium did not contain methionine. Furthermore, the addition of methionine to the selection medium resulted in a marked decrease of methyl mercury resistant mutants.

Eight mutants were crossed to normal strains, the diploid cells were sporulated, and genetic analysis was performed on the resulting meiotic segregants by standard procedures. In all eight cases, the methionine requirement segregated in the manner expected of a single chromosomal gene.

We then performed complementation tests with all pairwise combinations of the eight mutations. From this and from analysis of crosses among the mutants, it was established that all eight were allelic mutations. To determine whether the genetic lesions responsible for the requirement of methionine in the methyl mercury resistant strains arose at one of the previously identified genes concerned with methionine biosynthesis or whether they occurred at a previously undetected locus, complementation tests were carried out between this *met* gene and the standard methionine tester strains obtained from the Yeast Genetics Stock Center, University of California, Berkeley. These *met* mutants

complemented mutants in all the previously identified *met* genes represented in the stock collection, *met1*, *met2*, *met4*, *met5*, *met6*, *met7*, *met8*, *met10*, *met13*, and *met14*, as well as *met3* which was isolated in our laboratory. (As far as we know, the *met9*, *met11* and *met12* mutants do not exist or represent alleles of some of the above loci (see refs 13 and 14).) To rule out the possibility of interallelic complementation between our *met* mutations and any of the other known *met* genes, a methyl mercury resistant strain was crossed to each of the methionine testers individually. Tetrad analyses of spore clones from these crosses showed that the *met* mutations in our strains segregate independently of all available previously known genes involved in methionine biosynthesis. We have designated this new locus *met15*.

We could not demonstrate resistance by comparing growth of the normal strain and *met15* strains on media containing various concentrations of methyl mercury or containing gradients of methyl mercury. Perhaps the differences are too slight to be revealed by these methods. The resistance of *met15* strains to methyl mercury, however, can be unequivocally shown by a reconstruction experiment (Fig. 1).

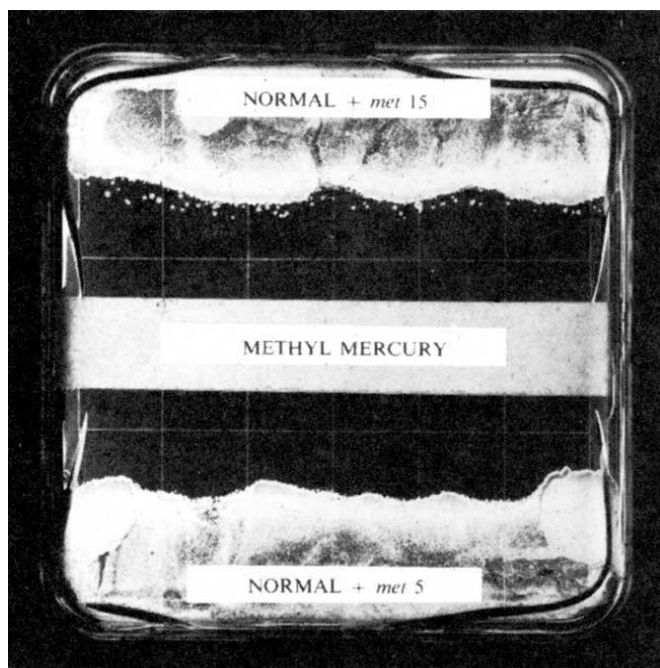


Fig. 1 Methyl mercury resistance of *met15* strains. The upper half of the plate was spread with approximately 2×10^6 cells from a mixed suspension of normal + *met15* strains and the lower half with 2×10^6 cells from a suspension of normal + *met5* strains. The ratio of normal to *met* cells in both cases was about 1,000:1. The paper strip in the middle was impregnated with $100 \mu\text{l}$ of 3×10^{-4} M solution of methyl mercury chloride. The photograph was taken after 7 d of incubation at 30°C . The methyl mercury resistant colonies of the *met15* strain (upper) can be seen growing before the front of the lawn of the normal strain.

The normal strain AS2-2C (α *leu2-1*) and the mutant S4 (α *leu2-1 met15-4*) obtained from AS2-2C were grown separately, washed and mixed. In addition a mutant S46 (α *leu2-1 met5*), directly isolated by replica plating techniques from AS2-2C, was similarly grown and mixed with the normal strain. The two kinds of mixed populations, as described in the figure legend, were plated on minimal agar medium supplemented only with leucine and exposed to methyl mercury. Only in the section of the plate seeded with normal + *met15* cells did any resistant colonies appear. These colonies could be isolated and analysed further. In one such reconstruction experiment 154 of the 164 isolated

colonies carried the *met15* gene, and more importantly, 149 of the 154 *met15* colonies carried the input allele, *met15-4*. The remaining five were apparently new mutations at the *met15* locus. The frequency of recovery of the input *met15* strain was markedly reduced if the experiment was done on a medium supplemented with leucine and methionine. In the experiment described above, only fourteen *met15-4* colonies could be recovered from the medium containing leucine and methionine. Thus methionine inhibited the expression of resistance, a phenomenon corroborated by the decrease in mutation frequency on methionine-supplemented medium.

An interesting property of the resistant mutants was uncovered when we examined the interrelationship between methyl mercury resistance and methionine auxotrophy by growing the mutants on double gradient plates of methyl mercury and methionine (Fig. 2). The gradients were generated by adding the methyl mercury and methionine on paper strips which were placed at right angles to each other along the edges of the plates. Figure 2 shows growth patterns of S4 (α *leu2-1 met15-4*) and S46 (α *leu2-1 met5*). As noted earlier, the two strains are isogenic except for their *met* genes. The methionine auxotroph (*met15*), which is resistant to methyl mercury, has a growth pattern very different from the other methionine auxotroph (*met5*). We have so far compared the growth patterns of five different *met15* mutants with those of controls consisting of strains carrying mutations at *met5*, *met8* or *met10* locus, but otherwise isogenic to the *met15* strains. In every case, the *met15* strain showed the growth pattern depicted in Fig. 2a, whereas the control exhibited the pattern represented in Fig. 2b. We interpret the peculiar growth response of *met15* strains on the double gradient plates to indicate that at a critical concentration of methyl mercury the methionine requirement of the *met15* mutants is partially or completely alleviated. It also appears from the growth pattern on the *met5* plate that methionine at higher concentrations may be preventing methyl mercury toxicity.

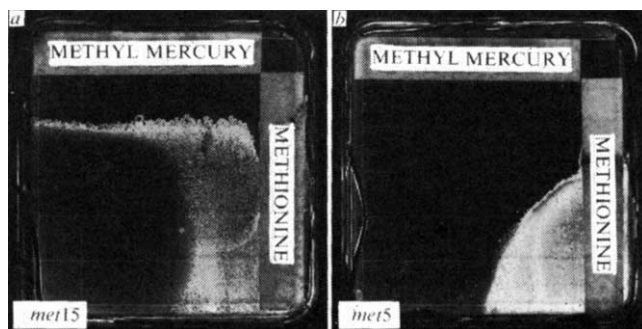


Fig. 2 Growth patterns of a *met15* (a) and a *met5* (b) strain on double gradient of methyl mercury and methionine. Approximately 1.3×10^6 cells per plate were spread uniformly over the whole area of the plates which contained minimal agar medium supplemented with leucine; $100 \mu\text{l}$ of 3×10^{-4} M solution of methyl mercury chloride and $200 \mu\text{l}$ of 1.6×10^{-2} M solution of methionine were used on the respective strips. The photographs were taken after 8 d of incubation at 30°C . The protruding area of growth of the *met15* strains indicates that the methionine requirement was completely or partially alleviated at certain concentrations of methyl mercury.

The data so far are consistent with the following interpretation of the interrelationship of methionine requirement and methyl mercury resistance. We hypothesise that a compound involved directly or indirectly in the biosynthesis of methionine can cause detoxification either by the formation of a complex with methyl mercury or by conversion of it to a volatile product. This compound accumulates in *met15* mutants and inhibits methionine biosynthesis. The diminution of the methionine requirement of the resistant mutants

in the presence of methyl mercury may then be explained by the destructive action of methyl mercury on this compound. Methionine may inhibit the production of this compound by repression or feedback inhibition which would explain the poor expression of the resistance in the presence of methionine. These yeast mutants do not seem to detoxify methyl mercury by the same mechanisms found in other mercury resistant organisms¹⁵⁻¹⁸. We are continuing the investigation in the hope of explaining the results at molecular level as well as to test our working hypothesis.

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Free and Occluded Virus, similar to *Baculovirus*, in Hepatopancreas of Pink Shrimp

VIRUSES or virus-like particles have been reported infrequently from cells of estuarine and marine organisms¹⁻⁴. Herpes-type viruses were described from an estuarine fungus³ and oysters⁴. Helical or rod-shaped viruses have not been reported from most aquatic invertebrates, although rod-shaped virus-like particles were reported from a micro-annelid⁵ and aquatic beetle⁶.

Recently, I observed rod-shaped virus particles and related inclusion bodies in cells of pink shrimp (*Penaeus duorarum*) that were experimentally exposed to the polychlorinated biphenyl (PCB), Aroclor 1254. This report describes these particles and demonstrates their similarity to certain non-inclusion viruses and to nuclear polyhedrosis viruses of the *Baculovirus* group previously described only from insects and mites⁶⁻¹⁰.

Adult pink shrimp, from the Gulf of Mexico near Cedar Key, Florida, were kept in flowing seawater and exposed to 3-5 $\mu\text{g l}^{-1}$ PCB for 30-52 d as described elsewhere¹¹. Control shrimp from the same stock were maintained in PCB-free flowing seawater. Non-experimental pink shrimp which were collected periodically from near Cedar Key and Pensacola, Florida, and prepared immediately for cytology are considered here as feral or wild shrimp. Hepatopancreatic tissues from forty exposed shrimp, forty control shrimp and fifty feral shrimp were fixed for histology in Davidson's fixative¹², sectioned and stained with Harris haematoxylin and eosin, mercury bromophenol blue or the PAS method.

Hepatopancreatic tissues from five exposed, five control and ten feral shrimp were fixed for electron microscopy

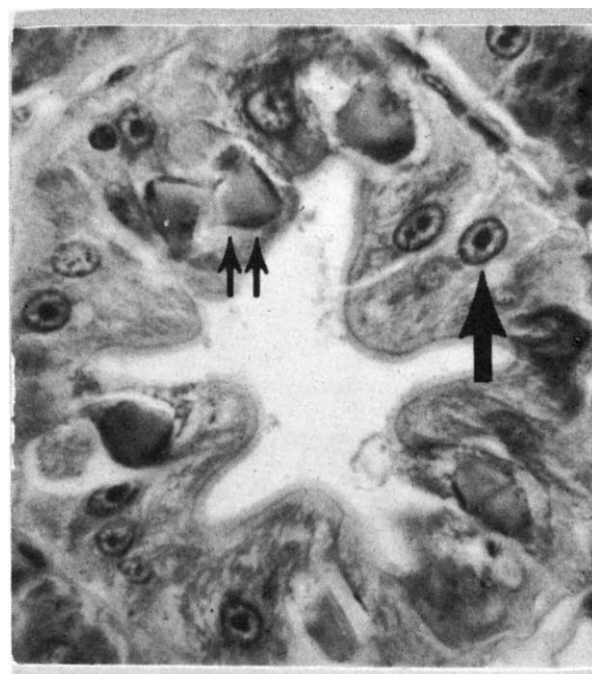


Fig. 1 Light micrograph of cross section of hepatopancreatic tubule from pink shrimp exposed to PCB; note normal nucleus of epithelial cell (one arrow), and crystalloid, tetrahedral inclusion bodies in hypertrophied nucleus of affected cells (two small arrows).

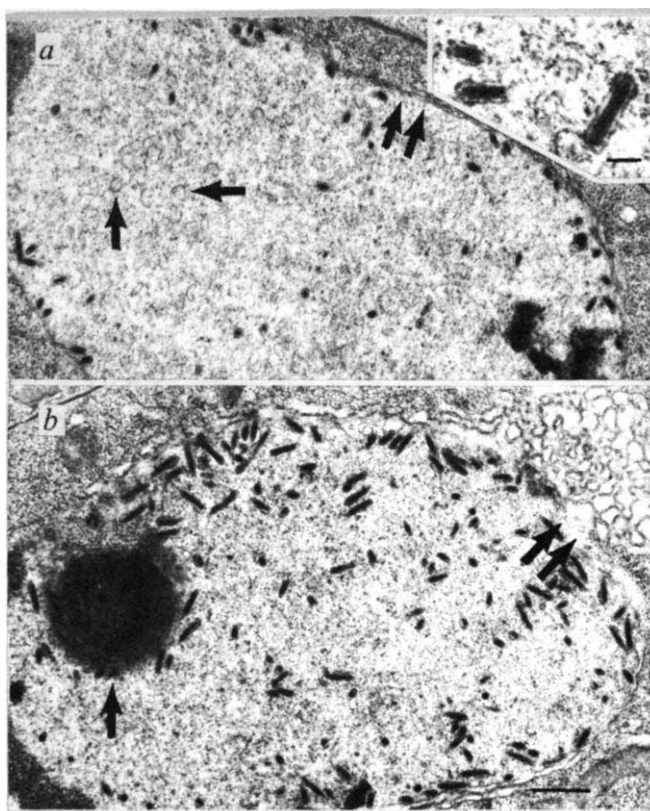


Fig. 2 a, Hypertrophied nucleus in epithelial, absorptive cell from hepatopancreas of shrimp exposed to PCB; non-occluded rod-shaped virions in longitudinal and cross section; single arrows point to intranuclear membranes associated with early virion formation, double arrows indicate abnormal multi-laminate nuclear envelope. Inset: high magnification of rod-shaped virion in nucleus (bar=0.1 μm); note single envelope surrounding nucleocapsid. b, Moderately heavily affected nucleus; virions distributed mostly around inner periphery of nucleus; arrow points to degenerate nucleolus; double arrows point to envelope membranes entering the cytoplasm. Note many free ribosomes in cytoplasm (bar=1 μm).

(2.5% glutaraldehyde and 1% osmium) and embedded in Epon 812. Sections 50–100 nm thick were cut, collected on unsupported 300 mesh copper grids and stained with uranyl acetate and Reynold's lead citrate according to the short method of Hayat¹³. Approximately 3,000 hepatopancreatic cell profiles from feral and control shrimp and 1,500 hepatopancreatic cell profiles from exposed shrimp were examined with the electron microscope.

Light microscopy of fresh squashes and fixed, stained hepatopancreatic tissues of control shrimp revealed normal histological and cytological structure. Thirty per cent of shrimp exposed to PCB, and approximately 20% of feral shrimp from near Cedar Key, however, had crystalloid inclusion bodies in distorted, hypertrophied nuclei of hepatopancreatic epithelial cells (Fig. 1). These nuclear inclusion bodies were usually tetrahedral (pyramidal) in form in fresh squashes and triangular in sections. They ranged in size from 0.5 μm to 12 μm from base to point, and stained light blue with mercury bromophenol blue, and were PAS-negative.

Electron microscopy demonstrated normal ultrastructure of hepatopancreatic tubule cells and nuclei in feral and control shrimp. However, approximately 8% of the nuclear profiles of hepatopancreatic tubule cells from three PCB-exposed shrimp were hypertrophied, and contained rod-shaped, free (non-occluded) and occluded virions (Figs 2–4). Light microscopy showed that these exposed shrimp had

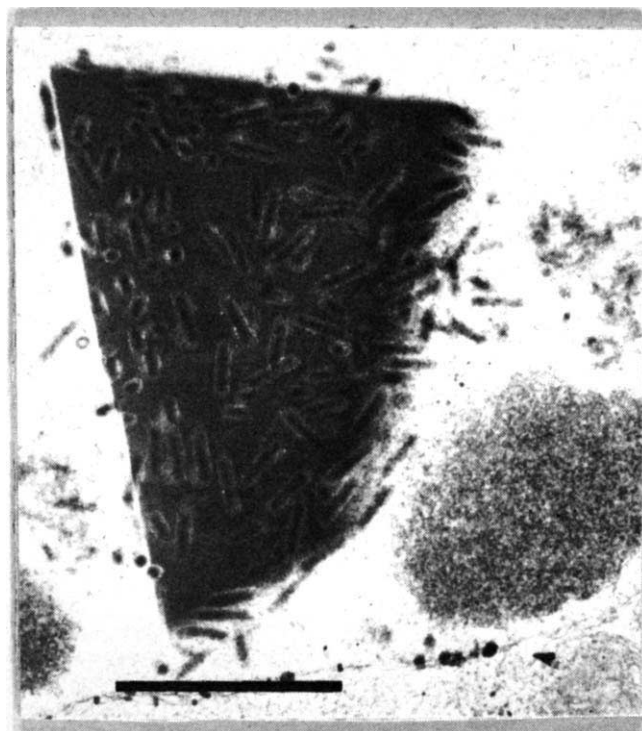


Fig. 4 Higher magnification (EM) of occlusion body from Fig. 3. Note virions occluded randomly within body. This body has a two-dimensional triangular form of crystalloid inclusion body observed with light microscopy in nuclei of hepatopancreatic cells; compare with Fig. 1 (bar = 1 μm).



Fig. 3 Low magnification electron micrograph of section through hepatopancreatic tubule from exposed shrimp. Note profile of epithelial cell and nucleus (one arrow) containing free virions and intranuclear, crystalline occlusion body (two arrows); note hypertrophy of affected nucleus (bar = 5 μm).

numerous tetrahedral, crystalloid inclusion bodies in nuclei of hepatopancreatic cells.

A typical free virion within a cell nucleus consisted of a rod-shaped, electron-dense nucleocapsid surrounded by an electron-lucid area enclosed by one or two envelopes (Figs 2a, inset, 2b and 5). Nucleocapsids varied in length (from 210 to 316 nm; average 288 ± 31 nm, $n=30$) but had a relatively narrow range of diameters (from 44 to 64 nm; average, $59 \text{ nm} \pm 6 \text{ nm}$, $n=30$). In cross section, the average diameter of nucleocapsids plus their containing envelopes was $75 \text{ nm} \pm 7.5 \text{ nm}$ ($n=30$). The envelopes surrounding the nucleocapsids (Fig. 5) seemed to be membranous with spatial and structural relationship to the nucleocapsid similar to that of envelopes in nuclear polyhedrosis virus (a *Baculovirus*) from *Bombyx mori*⁹.

Virions were distributed in most nuclei in greater numbers near the inner nuclear membrane (Fig. 2a and b); however, they were associated with or occluded in crystalline bodies

at the ultrastructural level in some nuclei (Figs 3 and 4). These crystalline, occlusion bodies were always triangular in section, paracrystalline in structure, and were the only structures observed with the electron microscope that could be related to the intranuclear, tetrahedral, crystalloid bodies observed by light microscopy (Fig. 1). The occluded virions were similar in dimensions and structure to the non-occluded virions.

Evidence suggesting early stages of virion formation was found in altered nuclei (20% of cell profiles from exposed shrimp) that contained few or no completely formed virions. U-shaped membranous arrays occurred in these hypertrophied nuclei (Fig. 2a). These arrays and associated immature virions were similar to certain stages in early virogenesis of *Rhabdionvirus oryctes* in the rhinoceros beetle⁷, and of the virus reported from the whirligig beetle⁶.

Ultrastructural cytopathic alterations were associated with the presence of virions. Nuclei that contained virions in large numbers were often 1.5–2 times greater (hypertrophied) in profile area than normal nuclei (Fig. 3). Their cytoplasm, however, usually exhibited no parallel difference in profile area. Heavily affected nuclei had a distinct loss of heterochromatin (Fig. 2a and b), and some affected nuclei had no heterochromatin. Nucleolar degeneration was evident in nuclei that contained many virions (Fig. 2b). The most remarkable change associated with the presence of large numbers of virions was a proliferation of nuclear membranes (Fig. 2a and b), and their eruption into the cytoplasm to form a multimembranous labyrinth (Fig. 2b). Cells whose nuclei contained many virions possessed cytoplasm filled with ribosomes, but without normal numbers of mitochondria or endoplasmic reticulum vesicles (Fig. 2a and b). Most of these changes have been observed in cells of insects that were infected by *Baculovirus*. At present it is not known if exposure of infected shrimp to PCB enhances the cytopathic effects of the virus.

The following findings suggest a close relationship of the shrimp virus to the *Baculovirus* group¹⁰ of invertebrate viruses. The virions were found in nuclei of hepatopancreatic cells of an arthropod (these cells are analogous in many aspects to fat body and midgut cells of insects). They are rod shaped (bacilliform), enclosed by one to two envelopes or capsids, found both free in nuclei and occluded in crystalline bodies within nuclei¹⁰, and were 288 nm long by 75 nm in diameter, thus falling within the size range of *Baculovirus*^{9,10}. Stromata which seemed to be virogenic were present in affected nuclei, and cytopathic alterations of affected cells and nuclei were similar to those reported for *Baculovirus* infections in insects.

In summary, a rod-shaped, free and occluded virus exists in a marine shrimp, indicating that marine crustacea are potential hosts for viruses similar to certain viruses infecting insects and mites. So far, the virus has been found only in shrimp taken from near Cedar Key and experimentally exposed to the toxic chemical, Aroclor 1254 (PCB). The virus probably is a natural parasite, however, previously undetected, of estuarine and marine shrimp.

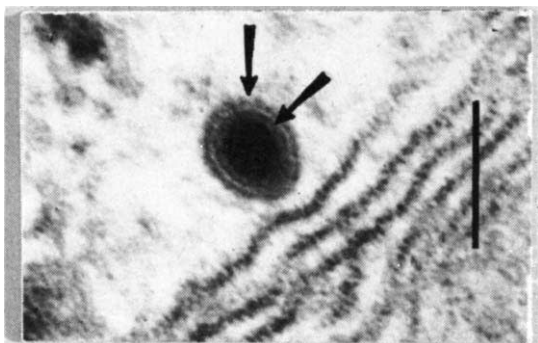


Fig. 5 Cross section of free virion in nucleus near abnormal, multimembranous (six membranes) nuclear envelope; note outer envelope and capsid of the nucleocapsid of virion (arrows) (bar=0.1 μ m).

Studies of possible interactions of the PCB and virus in pink shrimp may provide valuable information needed to clarify the relationship between natural infectious diseases and pollutant chemicals in the aquatic environment.

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Inhibitory Effects of Individual Components of the Sex Pheromone of a Tortricid Moth on the Sexual Stimulation of Males

RECENTLY, *cis*-9 and *cis*-11-tetradecen-1-ol acetates, the principal components of the sex pheromone of the smaller tea tortrix, *Adoxophyes fasciata*, and the summerfruit tortrix, *A. orana*, were isolated from virgin females, identified and synthesised¹⁻³. Individually, neither component was active even when presented in unusually large amounts. Furthermore, most male moths failed to respond when the two compounds were presented in sequence within a few seconds of each other. In contrast, a mixture of the two compounds at the 10^{-5} μ g level elicited the characteristic sexual response from the male moths. Some studies on sex pheromone perception have revealed that geometrical isomers strongly inhibit the response of male moths to their sex pheromone⁴⁻⁷. These facts prompted us to examine the effects of individual compounds and their geometrical isomers on the sexual behaviour of the smaller tea tortrix. We found that the individual pheromone components, but not geometrical isomers, had an inhibitory effect on male stimulation by the sex pheromone.

After synthesis, *cis*-9 and *cis*-11-tetradecen-1-ol acetates, and their geometrical isomers, were purified by thin-layer chromatography on AgNO₃-impregnated silicic acid and by gas chromatography on 15%-PEGA on Chromosorb W AW. The bioassay procedure was based on that of Tamaki *et al.*⁸, in which the mating dance of male moths was used as the criterion of response. The two compounds were assayed individually and in equimolar mixtures by applying them to pieces of filter paper and then exposing ten male moths of the smaller tea tortrix in 200-ml flasks. The quantities of each compound applied varied from 5×10^{-5} to 5×10^{-2} μ g. A minimum of four replications was used for each test.

Results are summarised in Table 1. Mixtures of the two compounds evoked a potent response of the male moths ranging from 74% at the lowest concentration tested (5×10^{-5} μ g of each compound) to 90% at the highest level tested (5×10^{-2} μ g of each compound). When presented individually, however, neither compound elicited the mating dance. *cis*-11-tetradecen-1-ol acetate presented 1 min after males had been exposed to the *cis*-9-isomer also failed to evoke more than a 4% response. Surprisingly, the mixture of *cis*-9 and *cis*-11-isomers also evoked no response when presented 1 min after previous exposure to more than 5×10^{-4} μ g of individual isomers. This mixture did elicit a potent response, however, from a group of male moths that had not been previously exposed to the individual compounds. In contrast, previous exposure to the geometrical isomers, *trans*-9 and *trans*-11-tetradecen-1-ol acetates, in concentrations equivalent to the pheromonal components, had no inhibitory effect on the sexual stimulation of males when exposed to the active pheromone.

Clearly the individual sex pheromonal components inhibit the subsequent stimulatory response of male moths of the smaller tea tortrix to the sex pheromone. The inhibitory effect of each pheromone component was apparent when there was an interval of only a few seconds between pre-exposure to one component and subsequent exposure to the mixture. The inhibitory effect was also confirmed by using virgin females. Suppression of mating was 90% and 60% in males pre-exposed for 1 min in 200-ml flasks to 10^{-2} μ g of *cis*-9-isomer and *cis*-11-isomer, respectively. The inhibitory effect of individual components of the sex pheromone on sexual behaviour of males of the smaller tea tortrix seems to be involved in the isolation of the smaller tea tortrix from any sympatric species that uses either *cis*-9 or *cis*-11-tetradecen-1-ol acetate as the sex pheromone. Although the olfactory

Table 1 Inhibitory Effect of Individual Pheromonal Components on the Sexual Stimulation of Male Moths of the Smaller Tea Tortrix *Adoxophyes fasciata*

Exposure stimulus *		Amount of each compound presented			
First exposure	Second exposure	$5 \times 10^{-5} \mu\text{g}$	$5 \times 10^{-4} \mu\text{g}$	$5 \times 10^{-3} \mu\text{g}$	$5 \times 10^{-2} \mu\text{g}$
		Percentage of male response †			
<i>cis</i> -9-TDA plus <i>cis</i> -11-TDA	None	74	82	80	90
<i>cis</i> -9-TDA	None	0	0	0	0
<i>cis</i> -11-TDA	None	0	0	0	0
<i>cis</i> -9-TDA	<i>cis</i> -11-TDA	2	4	0	2
<i>cis</i> -11-TDA	<i>cis</i> -9-TDA	24	4	4	0
<i>cis</i> -9-TDA	<i>cis</i> -9-TDA plus <i>cis</i> -11-TDA	5	0	0	—
<i>cis</i> -11-TDA	<i>cis</i> -11-TDA plus <i>cis</i> -9-TDA	20	0	0	—

* Duration of the first stimulus is one minute. TDA shows tetradecen-1-ol acetate.

† Criterion of male response is the 'mating dance'.

mechanisms of the moth are not yet understood, it seems that the male moths possess a complex mechanism that needs simultaneous receipt of the two components of the sex pheromone for release of subsequent sexual behaviour.

The present finding suggests a new approach for controlling the smaller tea tortrix by using only one component of the sex pheromone.

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Genetically Based Behaviour Patterns in *Drosophila melanogaster*

SOME time ago Bastock¹ demonstrated that yellow (y) mutant males of *Drosophila melanogaster* had reduced success in fertilising normal females. This tendency of females to reject y males provides a means by which variation in behaviour between populations might be easily studied.

The studies reported here involve four populations of *Drosophila*. A laboratory wild type (+), a y mutant strain, both obtained from Turtlox, and two wild type strains (No. 6 and No. 32) collected locally. The last two were selected from a larger collection of eight local populations, preliminary studies of which indicated a considerable variation in the rejection of y males by females. The four populations were studied with respect to the extent to which females rejected y males. The study was extended in an attempt to select a strain of females which would show a preference for y males. We are motivated by a general interest in the examination of genetically based behavioural differences between populations, and the extent to which such differences may be manipulated by selection.

The experiments described are based on the following

technique. A single adult virgin female, that had been kept for 2 or 3 d in a small tube with medium, was added, unetherised, to a vial containing medium and in which two males had been present for a similar period of time. Except where otherwise noted, one male was obtained from the same population as the female, and the other from the y mutant population.

Table 1 Choice of Males by Females

Female	Males	Success to y male	Success to other male	Unknown
No. 6	6, y	9	48	8
No. 32	32, y	6	137	21
+	+, y	3	43	19
y	+, y	164	208	18

Comparisons between the populations are shown in Table 1. The experiments were carried out at 25° C, and matings were observed for a period of 2 h from the time of the introduction of the female. Any matings not completed by that time are classed as 'unknown'. Significant differences with respect to the rejection of the y males exist between the two local populations, collected from areas approximately three miles apart ($P < 0.01$). The y females show the highest acceptance of y males. Clearly, however, no preference for y males exists in any of the populations.

Selection for enhanced acceptance of the y males was carried out as follows. Virgin adult female progeny were taken from one or more successful matings to a y male in the above experiments, and presented to a pair of males similar to those in the initial experiments. Thus both males are from the initial populations, while the female is from a cross between members of these populations. The next generation of females is again selected from successful crosses to y, and these females are again presented to males from the initial populations. Thus a No. 6 female mating with a y male gave rise to 6-1 females, and 6-1 females mating with y males gave rise to 6-2 females. Either one or two families were used for each generation; thus in each of the first two selected generations one family was used, while in generation 3, for strain 6, two families were used (6-3-1 and 6-3-2). 6-3-1 is derived from one of the two successes to y in the previous generation, and 6-3-2 from the other. Selection was only for females; the males were always obtained from the unselected parental populations. Results of the selection experiments for populations No. 6 and No. 32 are shown in Table 2.

It can be seen that no significant differences exist between the data for the first four generations for population No. 6; in fact the data do not differ significantly from that presented for No. 6 in Table 1. A marked change can be seen in the fifth generation where a preference for the y male can be seen; this preference is again seen in the sixth and seventh

generations. The rareness of successful matings to y by No. 32 females accounts for the fewer generations with respect to this population; the data suggest that this population may be less amenable to selection for successful matings with y males.

Table 2 Selection for Enhanced Preference for y Males

Female and generation	Males	Success to y male	Success to other male	Unknown
6-1	6, y	4	7	0
6-2	6, y	2	35	5
6-3-1	6, y	8	25	5
6-3-2	6, y	5	15	3
6-4	6, y	5	40	4
6-5-1	6, y	25	11	11
6-5-2	6, y	34	8	9
6-6-1	6, y	12	7	7
6-6-2	6, y	14	8	2
6-7-1	6, y	13	7	3
6-7-2	6, y	16	7	5
32-1	32, y	2	40	10
32-3	32, y	3	21	6
32-3	32, y	11	15	4
32-4	32, y	12	31	1
32-5-1	32, y	6	10	16
32-5-2	32, y	8	10	10

It should be realised that females of generations 6-5, 6-6 and 6-7 must contain large amounts of the y strain genotype, indeed parts of the y strain genotype must be present in all the selected females. Additional experiments in which the original pure y females are provided with a choice of No. 6 or y males show that the genotype of the pure y strain cannot alone account for the results in generations 6-5, 6-6 and 6-7 presented in Table 2. Thus, when the original pure y females were provided with a choice of No. 6 males or y males, sixty-one chose No. 6 males, twenty-seven chose y males and ten were unknown. It can be seen, then, that when pure y females are given the choice of No. 6 males or y males, the rejection of y males is even greater than when their choice is between the laboratory wild type and y males (see Table 1). The genetic basis of the preference for y males shown by selected females (Table 2) derived from the families 6-5-1,2, 6-6-1,2, and 6-7-1,2 must involve genes from both the original No. 6 strain and from the pure y strain, a further understanding of which must await a more detailed genetic analysis.

The results presented here are interesting in a number of respects. At present the simplest way of distinguishing between the two populations, No. 6 and No. 32, is by their behavioural differences described above. Although the y mutant is known to be associated with a single gene mutation, the genetic basis of the rejection of y males by females is unknown. It is possibly misleading to regard y males as offering a low level of stimulus, without carefully defining the mating system. We believe that use can be made of the genetic differences, present in female populations, of preference and rejection for y males, shown in the above experiments, in a closer examination of the female responses to male courtship signals.

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Microcapsules as Artificial Food Particles for Aquatic Filter Feeders

It has long been the aim of the aquaculture industry to replace natural foods with artificial diets which can be manufactured and stored, and there is an obvious need for the development of a successful artificial diet for aquatic filter feeders. Furthermore, if such diets are defined, they can be used to elucidate the exact nutritional requirements of the animals under culture. Provasoli and his co-workers^{1,2} have shown that *Artemia* can be reared in a sterile medium, but that a particulate phase is necessary to promote growth beyond the third stage metanauplii.

Artificial food particles are known to be acceptable to a wide range of filter feeders^{3,4}. In our experience, food supplied in a particulate form as egg slurry or minced fish⁵, or adsorbed on to the surface of particles, invariably leads to bacterial contamination with subsequent deleterious effects. An alternative approach is to use encapsulated diets in which the components are retained within an artificial membrane. The use of micro-encapsulation techniques in aquaculture has been advocated recently by Meyers and co-workers^{6,7} but, until now, no experimental results have been made available. The type of microcapsule that is required depends on the mode of feeding. In the case of molluscan larvae and adult bivalves, which ingest their food whole, the capsule wall should be impermeable and stable in seawater, but readily broken down by a change in pH or by the action of digestive enzymes. As far as we are aware, capsules of this type have not been described in the literature. Alternatively, for those larvae or adults which masticate their food, the capsule wall need only be broken mechanically to allow the contents to be ingested.

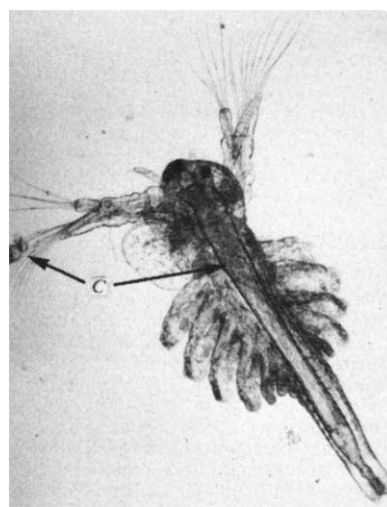


Fig. 1 Microcapsules fed to *Artemia*.

Microcapsules of this second type, with cross linked nylon-protein walls, can be prepared in the laboratory using a modification of the method described by Chang *et al.*⁸. 4,4'-Diamino-2,2'-biphenyldisulphonic acid (6 mmol l⁻¹) is added to give the membrane a resultant negative charge which reduces aggregation of the capsules. The concentration is twice that recommended by Chang *et al.*, to allow for the ionic effects of seawater and to increase the brittleness of the membrane. It was also necessary to double the concentration of sebacyl chloride (0.036 M) to produce satisfactory capsules in the right size range (20 to 80 μ m diameter). The capsule walls are easily ruptured by crustacean mouthparts and a wide range of water-soluble materials and particulate matter can be encapsulated to give almost neutrally buoyant capsules in a size range 5 to 200 μ m. The main disadvantage of this type of capsule is that

the nylon-protein wall is permeable to small molecules but the capsules can be used to produce defined macromolecular food particles containing starch and protein.

Various Crustacea have been observed feeding in suspensions of microcapsules and it has been shown that all the animals studied, *Macrobrachium* zoea, *Carcinus* zoea, late stage *Balanus* and *Elminius* nauplii, adult *Temora*, and *Artemia*, can rupture the capsule walls and ingest the contents, sometimes together with the crushed outer membrane. Figure 2 shows that, after 4 d, the percentage survival of *Artemia* fed on microcapsules containing 5% starch and 10% haemoglobin exceeded that of the starved controls. After 10 d, 25% of the fed *Artemia* had survived whereas all the starved animals had died. To obtain a mean value for 'growth' within the population of *Artemia*, we have used the growth index of Provasoli *et al.*² This is an arbitrary value related to size and the moult stage reached. The

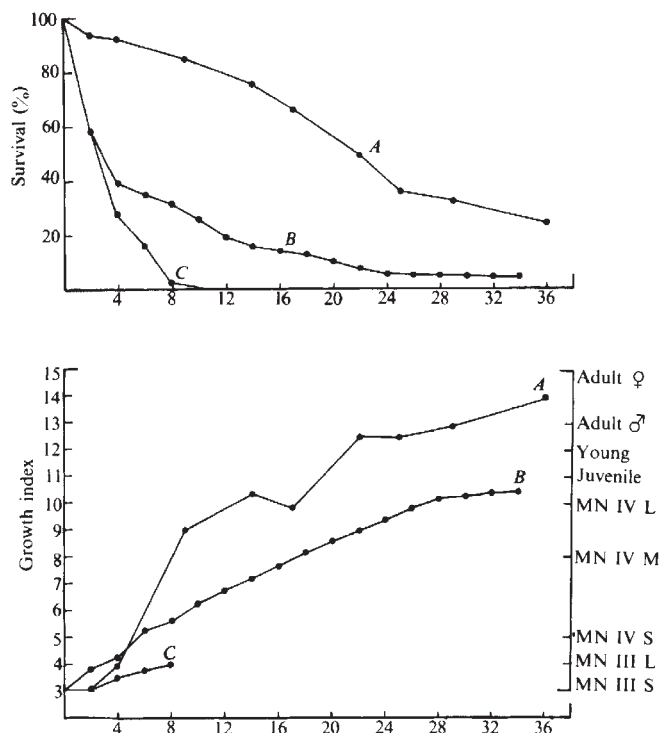


Fig. 2 Survival and growth of *Artemia*. Growth experiments were carried out at room temperature in beakers containing 600 ml of aerated seawater and 120 to 180 *Artemia*. The results are given as the means of two or three replicated experiments, B and C, and of a single experiment, A, with *Isochrysis galbana*. Microcapsules contained 5% soluble starch and 10% haemoglobin. The concentration of food particles was kept in excess of the daily intake for *Artemia* and the seawater and food changed each time the animals were counted. Penicillin G and streptomycin sulphate were added to the cultures at concentrations of 30 mg l⁻¹ and 50 mg l⁻¹ respectively. A, *Isochrysis*; B, microcapsules; C, starved controls.

base line is provided by the starved controls. Figure 2 shows that *Artemia* will not progress beyond the third metanauplius stage (growth index 4) without food¹. Those animals fed on microcapsules, and which survived, reached a growth index of 11 after 34 d with an average development to the juvenile stage although a few individuals reached the adult stage. Clearly, growth and survival was less than that achieved with *Isochrysis* but the encapsulated diet was incomplete and the starch:protein ratio was not optimal for growth of *Artemia*².

A further experiment was carried out to show that U-¹⁴C-labelled starch, contained in microcapsules, is assimilated by *Artemia* and the glucose carbon is incorporated into amino acid metabolism. Previous work with Crustacea⁹ has shown that carbon from U-¹⁴C-glucose is readily incorporated into alanine, glutamic acid, aspartic acid and glycine. Approximately 200 late metanauplius to young adult *Artemia* were fed with

microcapsules containing 5% starch (1 μ Ci mg⁻¹) and 10% haemoglobin for 48 h at 18° C in a litre of seawater. At the end of the feeding period the *Artemia* were removed and washed with isotonic ammonium formate solution, freeze dried and powdered in a micro ball mill. A sample of the dried *Artemia* was extracted with 5% trichloroacetic acid (TCA) and the insoluble protein residue oxidised with performic acid as described by Hirs¹⁰, and hydrolysed with 6 N HCl. The constituent amino acids were separated by one-dimensional thin-layer ion-exchange chromatography¹¹ and made visible by spraying with a ninhydrin reagent. Radioactivity was determined by scanning the TLC plate using a Berthold Thin-layer Scanner II with a slit width of 1 $\times$ 16 mm.

Figure 3 shows that glucose carbon from encapsulated U-¹⁴C starch is assimilated by *Artemia* and incorporated into the non-essential amino acids alanine, glutamic acid, aspartic acid and glycine resulting in ¹⁴C-labelled protein. No attempt was made to calculate the radiochemical recovery in the protein fraction except to note that the activity was low with a maximum count rate only twice the background count. A further sample of *Artemia* was extracted with chloroform-methanol and the neutral lipid classes separated by TLC on silica gel. Traces of ¹⁴C-label were detected in the fatty acid component.

It has been our intention to show that microencapsulation represents a feasible approach towards the development of artificial diets for filter feeders. Animals with very different food preferences, ranging from *Artemia* cultured on phytoplankton, to *Macrobrachium* zoea which feed on *Artemia* itself, were

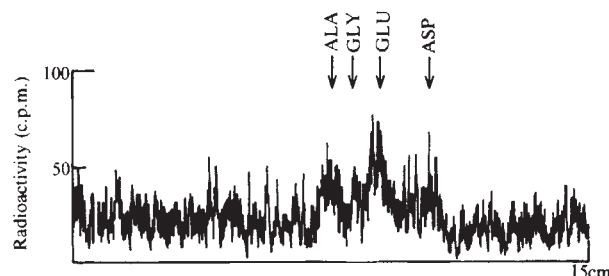


Fig. 3 Separation of constituent amino acids from protein hydrolysate of *Artemia*. Precoated Ionex-25 SA, Na⁺ form TLC plate (Macherey, Nagel and Co.) without pre-equilibration. Elution buffer, pH 3.3, sodium citrate, 0.4 N Na⁺, 0.4 M citrate at 50° C. Approximately half the total protein hydrolysate from 2 mg freeze-dried *Artemia* was applied to the plate as a 1 cm band and eluted to a distance of 15 cm. Individual amino acids were identified by comparison with a mixture of known standards.

observed to accept and ingest the contents of microcapsules provided these were supplied in the correct size range, 20 to 250 μ m. Although the ideal capsule should have an impermeable membrane which could either be ruptured mechanically or broken down by digestive activity, much can still be accomplished with the present capsules despite their permeability. Capsules containing starch and protein could be used as artificial food particles, together with natural food organisms, to provide essential nutrients such as vitamins. Lipids might also be adsorbed on to starch particles and incorporated into the capsules, which could then be used to determine the optimum starch:protein:lipid ratios for filter feeders. Metabolic studies in which ¹⁴C-labelled starch (or protein) is incorporated into capsules are already possible. Labelled fatty acids could be adsorbed on to starch particles as described by Morris *et al.*¹² and encapsulated for filter feeders. In addition, other metabolites or foreign compounds could be fed in an encapsulated form either adsorbed or attached covalently to a suitable high molecular weight carrier molecule.

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Degradation of *p,p'*-DDT in Reducing Environments

p,p'-DDD (*p,p'*-TDE; 1,1-dichloro-2,2-bis (*p*-chlorophenyl) ethane) is an extremely persistent environmental contaminant¹; it is a well known insecticide but is also derived from *p,p'*-DDT (1,1,1-trichloro-2,2-bis (*p*-chlorophenyl) ethane) in the environment by a process of reductive dechlorination. The conversion of DDT to DDD has been observed in a wide variety of biological systems, both living and dead². The mechanism by which this degradation of a highly chlorinated molecule takes place in the environment is of considerable interest but has not been clearly established^{3,4}.

We have recently re-examined the environmental degradation of DDT and have measured DDD/DDT ratios after exposing DDT to a variety of conditions: sterile and non-sterile; oxidising and reducing; and appropriate combinations. The systems examined were: (1) sewage sludge, (2) baker's yeast, (3) iron salts and iron porphyrins, (4) bacterial cultures and (5) annelid worms. The results from this set of conditions, which ranged over complex biological situations, pure cultures and chemical systems, have led us to the conclusion that the conversion of DDT to DDD in the environment is mediated by reduced iron porphyrins and is not an essential part of cell metabolism.

Secondary sewage sludge converts DDT to DDD under anaerobic conditions⁵⁻⁸, with a half-life of as little as 3 h, but DDD is itself stable. The data in Table 1 show that not only is this ability not destroyed by sterilisation with γ -radiation (Experiments 1 and 5) but that even after inactivation by aeration (Experiment 6) or by autoclaving⁵ (Experiment 3), activity can be restored instantly if the *Eh* is sufficiently lowered by addition of sodium dithionite (Experiments 4 and 7), a reducing agent widely used in biochemical studies⁹. This suggests to us that direct microbial metabolism does not account for the reductive dechlorination of DDT in untreated sewage sludge.

Table 1 Degradation of *p,p'*-DDT in Anaerobic Sewage Sludge

Exp. No.	Sterilisation	Reduction or oxidation	<i>Eh</i> (mV)	DDD/DDT	% ¹⁴ C extracted
1	—	—	-285	0.8	71
2	—	Dithionite	-660	2.0	63
3	Autoclaved	—	-210	0.1	84
4	Autoclaved	Dithionite	-640	2.3	76
5	5 Mrad γ	—	-220	1.0	56
6	—	Aerated	+50	0.1	71
7	—	Aerated, then dithionite	-645	1.7	57

Aliquots (10 ml) of anaerobic sewage sludge were sterilised (15 pounds per square inch, 15 min or ⁶⁰Co source at 0.45 Mrad h⁻¹) and reduced (50 mg sodium dithionite in 0.5 ml water) or oxidised (rapid aeration for 90 min at 20° C) as indicated. ¹⁴C-*p,p'*-DDT (107 μ g, 0.45 μ Ci) in ethanol (0.1 ml) was added to each sample and the mixtures incubated (37° C, 90 min). The sludge was obtained from the anaerobic digester of the Bristol Corporation Sewage Treatment Works, and was concentrated by decantation to 5% dry weight solids. ¹⁴C-*p,p'*-DDT (98% pure) was obtained from the Radiochemical Centre, Amersham, and unlabelled *p,p'*-DDT from Aldrich (99% pure). *Eh* measurements were made with a platinum spear electrode, a calomel reference electrode and a Philip Harris pH and Redox meter: readings were taken 1 min after the electrodes were inserted into the sample and the platinum spear was flame-cleaned between each determination. The samples were extracted (hexane: isopropanol 1:1, 3 $\times$ 10 ml), the combined hexane layers were analysed by GLC as previously described^{6,8} and by scintillation counting. DDD/DDT ratios are calculated directly from peak heights. Aqueous dithionite alone yielded only a small amount of DDD (see Table 3). Results shown are the mean of duplicate determinations. The DDT and DDD together account for 30–60% of the extracted ¹⁴C. More polar products, similar in chromatographic behaviour to those noted by Miskus *et al.*¹³, account for the remainder of the label in these and in the relevant experiments quoted in Tables 2 and 3.

A readily available microbial system is baker's yeast in which Kallman and Andrews¹⁰ have reported substantial conversion of DDT to DDD over periods of several days. In our experiments, however (Table 2), no significant reaction took place in fresh yeast suspensions in 1.5 h at 37° C unless the *Eh* was lowered by addition of sodium dithionite. Previous autoclaving brought a marked enhancement of this effect, suggesting that autoclaving released a chemical factor which at the appropriate *Eh* was an effective reducing agent for DDT.

Various putative reductants for the DDT to DDD conversion have been put forward. For example, Glass¹¹ claimed that a ferrosulfuric-ferrous couple was effective at high pH, but we achieved only a very slow reaction. Increasing the ferrous ion concentration in sewage sludge did not significantly increase the ability of the sludge to degrade DDT

Table 2 Degradation of *p,p'*-DDT Yeast Cell Suspensions

Sterilisation	Reduction	<i>Eh</i> (mV)	DDD/DDT	% ¹⁴ C extracted
—	—	+165	0.1	91
—	Dithionite	-510	0.4	91
Autoclaved	—	+50	0.1	97
Autoclaved	Dithionite	-600	1.3	91

Aliquots (10 ml) of an aqueous 10% w/v suspension of commercial yeast (Distillers, Yeast and Food Division) were used. The materials and procedure were otherwise the same as described under Table 1.

to DDD, nor did it restore this ability to autoclaved sludge. Addition of ethanol to increase the solubility of DDT in the presence of ferrous ions also failed to increase the extent of reaction. Castro¹² reported the rapid oxidation of a reduced iron porphyrin by DDT in isopropanol:acetic acid (1:1) saturated with KCl under nitrogen. Later, Miskus *et al.*¹³ suggested that reduced porphyrins might be the factor responsible for conversion in biological systems. This attractive suggestion has been reiterated^{5,14} and cytochromes

and other protein-bound haem compounds have since been shown to degrade DDT slowly under reducing conditions¹⁵. Using haematin dissolved in dilute sodium hydroxide ($pH > 11$) Miskus *et al.*¹³ obtained 10% conversion of DDT to DDD in 4 h with sodium dithionite as a reducing agent. Solubility is a problem in conducting experiments with Fe(II) porphyrins in the pH range normally associated with biological systems. Haematin (the Fe(III) porphyrin corresponding to the Fe(II) porphyrin, protohaem) is soluble in aqueous 0.1 N Na_2CO_3 but precipitated when it was reduced to protohaem by the addition of sodium dithionite. The addition of a detergent or ethanol prevented this precipitation and the results we thus obtained with protohaem and DDT are shown in Table 3. The conversion of DDT to DDD can therefore be accomplished rapidly and in a catalytic manner by an Fe(II) porphyrin at a pH frequently associated with biological systems (Table 3; Experiments 1–3). Rapid degradation of DDT was also obtained when ferrous sulphate was substituted for sodium dithionite (Experiment 4), but no significant degradation was observed with ferrous sulphate under conditions similar except for the absence of haematin. The addition of haematin and detergent to anaerobic sewage sludge greatly increased the DDT to DDD conversion (Experiments 7–10).

Table 3 Reaction of *p,p'*-DDT with Reduced Haematin

Exp. No.	Haematin (mg)	Tween 80 (mg)	Ethanol (ml)	Reducing agent	DDD/DDT	% ¹⁴ C extracted
1	0.6	—	—	Dithionite	0.25	54
2	0.6	25	—	Dithionite	0.83	42
3	0.6	—	1.0	Dithionite	2.7	84
4	0.6	25	—	Ferrous sulphate	1.9	28
5	—	25	—	Dithionite	0.09	46
6	—	—	1.0	Dithionite	0.06	95
7	—	—	—	Sludge	0.21	96
8	0.3	—	—	Sludge	0.36	85
9	—	200	—	Sludge	0.85	80
10	0.3	200	—	Sludge	3.5	81

DDT (3.55 mg, 0.1 μ Ci in Experiments 1–6; 1.78 mg, 0.05 μ Ci in Experiments 7–10) in ethanol (0.2 ml and 0.1 ml respectively) was added to each mixture of haematin (Koch-Light Laboratories), Tween 80 (Hopkin and Williams), ethanol, 0.5 ml 0.1 N aqueous Na_2CO_3 solution, and chemical reductant (25 mg) or sludge (10 ml). The source of the DDT and the incubation and analytical procedures were as described for Table 1, except that in Experiments 1–6 products were extracted into 10 ml ether by very vigorous agitation. The DDT : haematin molar ratio was 10 : 1.

The foregoing chemical experiments demonstrated that DDT to DDD conversion was effected in any situation in which DDT and reduced iron porphyrins were brought together in solution.

Reports have appeared and continue to appear^{4,10,16–20} reporting organisms which either do, or do not, readily metabolise DDT but they do not, mostly, purport to show the use of DDT as a sole carbon source. Our next experiment simulated the natural exposure of DDT to porphyrins, or porphyrin-like compounds, contained in microorganisms, at various oxidation/reduction potentials.

Bacteria derived from anaerobic estuarine sediments were grown in a chemostat on a medium containing about 10 p.p.m. DDT. Dilution rates down to 0.25 h^{-1} were used, gassed either with air to maintain aerobiosis or with nitrogen. We failed to observe any degradation of DDT under these conditions of continuous growth. But when the chemostat was turned off, DDT conversion was soon observed in the culture vessel and also occurred in the effluent reservoir where there was considerable autolysis.

In another experiment, pure cultures of twelve species of

bacteria from ten genera were grown aerobically on nutrient agar overnight and collected, and aqueous suspensions containing about 10^9 viable cells ml^{-1} were incubated with 10 p.p.m. DDT, with and without sodium dithionite, at 37° C for 1.5 h. Conversion of DDT was significant only in cell suspensions containing dithionite and was enhanced by using cell suspensions which had been previously heated at 100° C for 10 min.

In a laboratory experiment²¹ individuals of *Arenicola marina* (lugworms) living in well-aerated artificial burrows containing 2,000 p.p.m. of DDT were unable to degrade ingested DDT. But 48 h after death, when lysis and decay were well established, about 70% of the incorporated DDT was found to be converted to DDD and other products, confirming similar reports^{1–3,14} of degradation by dead animal tissues in anaerobic conditions or where no precautions had been taken against bacterial decay.

We believe that the foregoing rationalisation and extension of existing data bearing on the reductive dechlorination of the normally highly persistent DDT molecule has considerable environmental significance. Most living material contains iron porphyrins bound with protein in complex molecules such as haemoglobin, cytochromes and catalases. Decay should release iron porphyrins²² which, under the reducing conditions pertaining in the anaerobic environments characteristic of dead and decaying matter, would catalyse rapid conversion of any available DDT to DDD and other products. In terms of the breakdown of the total world mass of DDT this pseudo-biological mechanism could appreciably affect the environmental half-life, since it would come into operation whenever the DDT encountered the appropriate situation in passing through the food web. Anaerobic microenvironments are ubiquitous and include the sulphide biome of reducing sediments²³, the decaying cell contents of dead organisms and the lower portions of the alimentary tract of animals. The operation of such a process should result in high DDD/DDT ratios in the tissues of animals scavenging on dead and decomposing organisms.

Work is in progress to identify the other products formed in the reaction of DDT with reduced iron porphyrins and to seek evidence for the occurrence of this reaction in various environments.

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Freezing resistance in winter flounder *Pseudopleuronectes americanus*

THE blood serum freezing points of most marine teleosts are within the range of -0.5 to -0.8°C ¹. As large regions of the oceans have temperatures as much as 1°C below this range², many marine fish must either increase the osmotic concentrations of their blood or exist in a supercooled state. The latter is unstable and in the presence of ice, supercooled fish will freeze and die.

The winter flounder, *Pseudopleuronectes americanus*, inhabits the coastal waters of eastern North America from Virginia to Labrador³. In the northern part of their range they occur during the winter in large numbers in ice-covered shallow bays⁴. Using a freezing point osmometer (Fiske) Percy⁵ found that the blood serum of winter flounder from the Mystic River Estuary, Connecticut froze at -0.63°C during summer and at -1.15°C in winter. NaCl accounted for 83% of the freezing point depression in summer, but only 57% in winter. The solutes responsible for the remainder of the depression in winter fish were not identified. Umminger⁶ investigated freezing resistance in winter flounder taken from the same area. Using data obtained with a vapour pressure osmometer he concluded that the serum freezing points of winter fish were not unusually low (-0.71°C).

temperature at which the flounder could survive in the presence of ice was $-1.4 \pm 0.1^{\circ}\text{C}$.

In the serum of the Antarctic fish, *Trematomus borchgrevinkii*, there is a group of organic 'antifreeze' compounds (glycoproteins) responsible for the serum's unusually low freezing point⁷. These glycoproteins lower the freezing point of water without affecting the melting point by coating the surface of ice crystals, thereby removing them as possible nucleation sites^{8,9}.

To determine whether freezing resistance in the winter flounder is based on a similar mechanism, the freezing-melting behaviour of their serum was studied.

The freezing and melting points of serum samples were determined by placing the serum in small capillary tubes which were sealed at one end and plugged with mineral oil at the other. A small seed crystal was formed in the sample by spray freezing and the sample immersed in a refrigerated viewing chamber. The temperature of the chamber was controlled to within $\pm 0.01^{\circ}\text{C}$ and the seed crystal (0.25 mm diameter) viewed through a microscope. The temperature of the bath was raised or lowered 0.02°C per 5 min. The temperature at which the seed crystal disappeared was taken as the melting point and that at which the crystal grew as the freezing point. For an NaCl solution, the temperatures of melting and freezing are within 0.02°C of each other. For the serum of winter flounder collected in March, however, the freezing and melting points are widely separated (Table 1). This difference has been termed thermal hysteresis. Serum sodium and chloride ion concentrations accounted for 58% of the serum freezing point depression (-1.37°C) of March flounder and 94% for serum from fish collected in August when the water temperature was 17°C (serum freezing point = -0.69°C).

Dialysis showed that the major portion of the freezing point depression not due to NaCl in March fish was associated with the colloidal fraction of the serum. After exhaustive dialysis (tubing of molecular weight cutoff 3,500 daltons) against distilled water both the dialysed serum and the dialysate were returned to the original volume. Table 1 shows that the solutes responsible for the thermal hysteresis were unable to pass through the dialysis tubing and must therefore have molecular weights greater than 3,500 daltons.

These antifreeze compounds were stable at a temperature of 100°C and soluble in 10% trichloroacetic acid (TCA). Therefore, a crude preparation of the antifreeze was made by dialysing the serum and boiling it for 10 min followed by centrifugation at 15,000g for 20 min. The supernatant was diluted (1:10) with TCA and again centrifuged. After the

TABLE 1 Freezing point, melting point and inorganic constituents of whole and dialysed serum from *Pseudopleuronectes americanus*.

Specimen and environmental parameter	Freezing point ($^{\circ}\text{C}$)	Melting point ($^{\circ}\text{C}$)	Melting point — freezing point ($^{\circ}\text{C}$)	Sodium (mM l ⁻¹)	Chloride (mM l ⁻¹)	% Δ due to NaCl
Winter flounder serum						
March; water -1.2°C (12)	-1.37 ± 0.31	-0.75 ± 0.03	0.62 ± 0.35	250 ± 12	178 ± 6	58.1
Winter flounder serum						
August; water $+17^{\circ}\text{C}$ (8)	-0.69 ± 0.07	-0.67 ± 0.07	0.02 ± 0.001	194 ± 6	147 ± 14	94.0
Winter flounder						
March; water -1.2°C						
Dialysed serum	-0.65	-0.01	0.64	—	0.0	
Dialysate	-0.72	-0.70	0.02	—	172	

During the winters of 1972 and 1973 we investigated the freezing resistance of winter flounder collected by trawl at depths of 3 to 10 m in Head Harbor of St Margaret's Bay, Nova Scotia. Specimens were collected in March, a few days after the ice had begun to break up, when the water temperature was -1.2°C . The lower incipient lethal temperature (freezing temperature) determined by recording the lowest

TCA was removed from the supernatant by dialysis, lyophilisation resulted in a fluffy white residue. The freezing and melting points of aqueous solutions of this antifreeze preparation were determined at several concentrations (Fig. 1). The difference in freezing and melting points varied with concentration.

The discovery of thermal hysteresis in winter flounder

serum shows that the lowering of the freezing point of water by the antifreeze is not a colligative effect. In view of this, the freezing points reported by Umminger⁶, which were derived from vapour pressure measurements, would not be expected to agree with those reported by Pearcey⁵ as the vapour pressure of solutions is a manifestation of the number of dissolved particles. The serum freezing points (-0.71°C) observed by Umminger do, in fact, agree with the melting points we have obtained (Table 1). Freezing points measured with a freezing point osmometer are in reasonable agreement with those determined with the capillary technique. It is clear that in studies of freezing resistance, careful consideration should be given to choosing a method for determination of freezing points. It seems that visual examination of crystal growth under a carefully controlled temperature regime is the best method and is biologically the most meaningful because it agrees with the organismal freezing point.

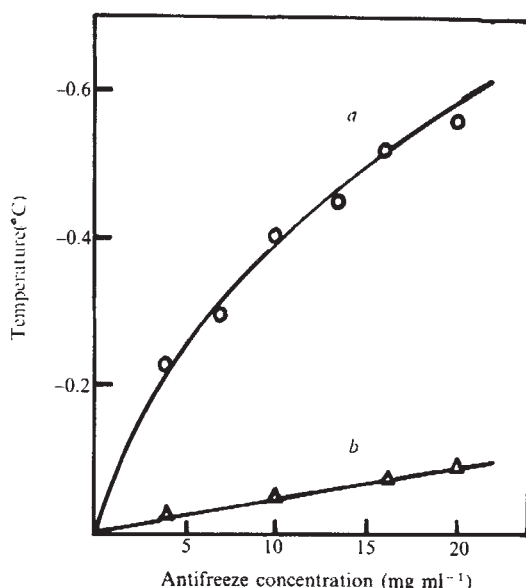


FIG. 1 Comparison of the freezing and melting points of aqueous solutions of antifreeze isolated from the blood serum of *Pseudopleuronectes americanus*. $\circ$, Temperatures of ice crystal growth in the presence of a seed crystal (freezing point); Δ , melting points of the seed crystal.

The freezing behaviour of the serum and partially purified antifreeze solutions was also studied by the use of freezing curves. The freezing points obtained with this technique agree with those obtained from the capillary method (Fig. 2 and Table 1). The shape of the freezing curve of the serum of the Canadian plaice, *Hippoglossoides platessoides*, which does not produce an organic antifreeze, resembles that of NaCl because the principal solute responsible for the depression of the freezing point is NaCl. The shape of the curve of NaCl and the plaice serum results from the fact that as freezing proceeds pure water freezes out leaving behind a more concentrated salt solution with a lower freezing point. Thus, during freezing the temperature of the sample remains slightly warmer than the bath temperature until all the solvent freezes—at which time the sample temperature rapidly approaches that of the bath. The shape of the freezing curve of the serum of the flounder differs from that of the plaice by the presence of a slight flat region near the freezing point. The flatness is more pronounced in the freezing curve of a solution of flounder antifreeze and much more so in a solution of the glycoprotein antifreeze from the Antarctic fish. The long plateau region of the latter results from incorporation of the glycoprotein into the solid phase

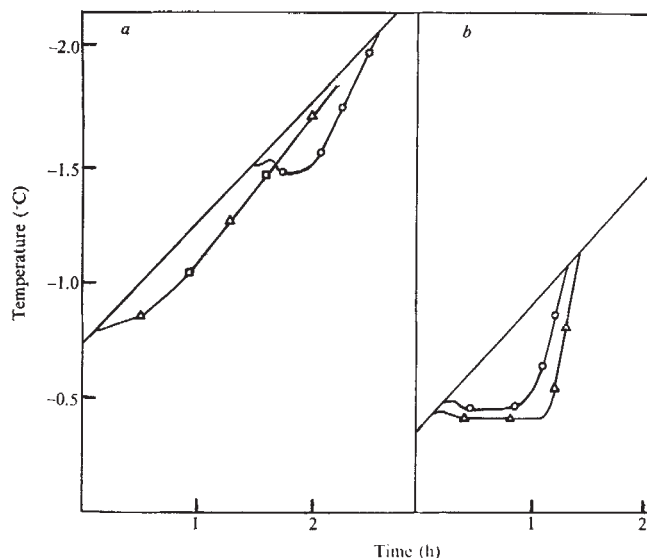


FIG. 2 a, Freezing curves of an aqueous 220 mM NaCl solution (Δ); of the blood serum of the Canadian plaice, *H. Platessoides* ($\square$); and winter flounder, *P. americanus* ($\circ$). b, Freezing curve of a solution of antifreeze (10 mg ml^{-1}) isolated from the serum of *P. americanus* ($\circ$); and of a solution of antifreeze (10 mg ml^{-1}) isolated from the blood serum of the antarctic fish *Trematomus borchgrevinkii* (Δ). The freezing point obtained from a freezing curve is the temperature where the sample temperature deviates from the bath temperature—indicating a warming of the sample resulting from the latent heat of fusion.

during freezing. The glycoproteins are equally partitioned between the liquid and solid phases which indicates they depress the freezing point of water by coating the surface of seed ice crystals, thereby preventing water molecules from joining the ice lattice⁷⁻⁹. Although no measurements have been made to show that the flounder antifreeze is equally partitioned between the liquid and solid phases during freezing, the flatness of its freezing curve strongly suggests that this is the case.

The thermal hysteresis and the freezing curve data lead to the inference that the mechanism of action of the flounder antifreeze is similar to that of the glycoproteins in Antarctic fish. Acrylamide gel electrophoresis and amino acid analysis indicate that the antifreezes of the two fishes differ markedly in composition. Therefore, it seems that distantly related families of cold water fishes have adapted to freezing conditions by evolving different large molecular weight compounds which, however, possess similar antifreeze activity.

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book reviews

Loving only the dead

Ten Years after Ivan Denisovich. By Zhores A. Medvedev. Translated from the Russian by Hilary Sternberg. Pp. xiv+202. (Macmillan: London and Basingstoke, November 1973.) £3.95.

THIS severely factual book, by a distinguished Soviet biologist temporarily working in England, encompasses three themes: the official persecution of Alexander Isayevich Solzhenitsyn; the elevation of the Big Lie to an instrument of policy; the ability of a profound creative writer who is also a fearless patriot to console the wounded spirit of his fellow citizens, oppressed not only by arbitrary cruelty but also by Universal Silence. For the reader in the West, Solzhenitsyn's novels are documentary accounts of reality distilled through the writer's imagination, and thereby an aesthetic experience: for the Soviet reader, they are the healing voice of truth, of great evil admitted, faced and endured.

Medvedev is a 'dissident' who has deserved well of his fellow Soviet citizens by disseminating, in *samizdat*, his detailed account of the official suppression of scientific genetics in the Soviet Union at the behest of the vicious charlatan, Lysenko. Solzhenitsyn wrote to Medvedev to encourage him when he was attacked for his factual writings about Lysenko, and their friendship stemmed from this act of support. The book under review makes it very clear how vital such acts of support by compatriots are to a Soviet citizen who is at odds with the authorities, and his recognition raises difficult questions for us in the West, who must decide whether our words of support are a help or a peril.

In his new book, Medvedev describes the circumstances of the publication in 1962 of *One Day in the Life of Ivan Denisovich* (on the express authority of Khrushchev who was then still concerned with surreptitiously blackening Stalin's memory); the first signs of official disapproval, when Solzhenitsyn was passed over for the Lenin Prize and had the manuscript of *The First Circle* and other papers confiscated; the prolonged but frustrated attempts by the remarkable literary editor, Tvardovsky, to publish *Cancer Ward* in his journal *Novy Mir*; the attempt to turn Solzhenitsyn into a non-person at the Fourth Writers' Congress; his expulsion from the Writers'

Union and that of Tvardovsky from his editorship; the prolonged official chicaneries in connection with Solzhenitsyn's Nobel Prize, which in the event was never presented in person; and, running like a refrain through the whole book, the many attempts by officialdom to blacken Solzhenitsyn's character at home and abroad by Big Lies about his wartime record, social background, daily habits, alleged support of Russian émigré organisations, by untruthful articles about his unpublished confiscated novels, unauthorised publication of a juvenilia disowned by Solzhenitsyn, and so on. Apart from that, Medvedev also quotes numerous specific instances of Solzhenitsyn's friends and admirers who lost their membership in the Party or Writers' Union, or their jobs, for either supporting Solzhenitsyn or for opposing or traducing him when told to do so by authority.

All this is presented in Medvedev's characteristic style of dry and scrupulous precision. (Readers who recall the detailed circumstantial evidence for postal censorship in his earlier book, *The Medvedev Papers*, will recognise the style.) A whole chapter is here devoted to the account of a determined detective exercise which eventually proved that mendacious articles about Solzhenitsyn published in two obscure periodicals in Italy and Poland had in fact originated in Moscow. Medvedev throughout evinces two predominant concerns—for accuracy and for the law. This is evident from the first page: the preface explains the circumstances in which this book was published, connected with Medvedev's concern not to offend against the new restrictive Soviet laws on publication abroad by Soviet authors, consequent on the Soviet Union's signature of the International Copyright Convention. In many places in the book, he explains in detail why various official acts against Solzhenitsyn were not only oppressive but also illegal. It seems that the dread censorship itself, Glavlit, is strictly illegal. It is impressive and indeed encouraging that Soviet 'dissidents', Medvedev notable among them, and unlike so many dissidents in the West, observe their country's laws more scrupulously than their own government. When, one distant day, the laws of the Soviet Union become more liberal, one may hope that

they will still be obeyed by the citizens and perhaps even by the government.

Medvedev's skills do not include evocative portraiture. Solzhenitsyn and Tvardovsky do indeed come alive in the book, but only through their own deeds and words, carefully related; Medvedev does not attempt set descriptions or indeed flights of literary criticism, though he does quote verbatim a few choice pieces of official 'criticism'. But then, this book is concerned with history, and he leaves literary analysis to others suitably qualified. A number of Russians who befriended Solzhenitsyn, such as the physicists Kapitsa and Pavlinchuk and the cellist Rostropovich, are briefly sketched, but again their personalities emerge through their reported deeds.

The book quotes the entire text of Solzhenitsyn's message to the Fourth Writers' Congress which he was prevented from attending. This is an eloquent attack on censorship and the personal oppression of individual writers. He points out that once a troublesome writer is dead, eventually his works find their way back to the Soviet public; Pasternak is a case in point. Solzhenitsyn quotes Pushkin with heavy irony: "They are capable of loving only the dead". He might have added the bitter words of Auden: "Let us praise while we can The Vertical man, Though we honour none But the horizontal one". But one must face the question; when we in the West praise the vertical man and condemn his oppressors, those who constantly strive by persecution, imprisonment and starvation to turn him into a horizontal one—do we help or do we hinder, do we protect or do we endanger? Understandably, Medvedev does not address himself to this question, and during a recent televised discussion when this question was tangentially debated, he maintained a dignified silence. Let us see what advice Solzhenitsyn himself has for us.

In the *New York Review of Books* of October 4, 1973, there is a transcript of a recent interview granted by Solzhenitsyn. He has this to say: "It must be understood that the East is not at all indifferent to protests from Western society. On the contrary it mortally fears them—and only them; but only when such protest is expressed by the strong and unified voices of hundreds of eminent people, by the opinion of an entire continent. Only then can an 'advanced' regime be

shaken. But when protests are timid and isolated, made without faith in their success and accompanied by the inevitable reservation that 'it is also the same in Greece, Turkey and Spain', they provoke only laughter from our oppressors. . . . The West by its publicity has already done much to help and save many of our oppressed . . .'. And the first of the modern dissidents, Pavel Litvinov and Larissa Daniel, in 1968 explicitly addressed their particular protest to world public opinion, adding that to address their own government and news media would be useless.

Such a message presupposes a response. And on the nature and indeed the wisdom of such a response, opinion in the West is deeply divided. Whether the victims are scientists or ballet dancers who wish to emigrate to Israel, writers who cannot publish at home, musicians who cannot perform abroad, some wish to follow Solzhenitsyn's fierce and explicit call, others prefer private pressure through private channels. Since such pressure must necessarily remain secret, the rest of us cannot form an opinion of its efficacy. Those who are in a position to exert private pressure and prefer it are right to follow this path, so long as they do not seek to warn off the grass others who see hope in a public approach. These still have to make the difficult decision whether or not to mention names, to specify the individual they are seeking to help. One must make one's own decision in each instance: perhaps fame is a form of safety, so that a notable figure will be helped by a protest emanating from a group of other notable figures (as Solzhenitsyn indicates, they need to be eminent), while an unknown victim, if named, may well suffer the more. We cannot know for sure, but when distinguished men like Solzhenitsyn, Sakharov and Levich all offer the same urgent advice, we had better heed it. Some differ: Roy Medvedev, Zhores' brother, has criticised Sakharov and Solzhenitsyn for calling for pressure from the West, and Sakharov in turn has just issued a statement regretting this, and maintaining his advice. Opinions do conflict among the victims, but this does not absolve each of us from the necessity of facing the issue and reaching a decision. Whatever their decisions, everyone with a concern for the future of Soviet society should read Zhores Medvedev's book.

ROBERT CAHN

Human chromosomes

Human Chromosomes. By E. H. R. Ford. Pp. xiii+381. (Academic: New York and London, April 1973.) £7.

DR FORD has written a book on human chromosomes which should be useful for students in the important and ex-

panding field of medical genetics. The first third of the book includes well illustrated descriptions of mitosis and meiosis and of the practice of karyotyping. This is followed by an outline of molecular genetics and of chromosome structure leading to a discussion of dosage compensation and of the origin of chromosomal abnormalities. The last and strongest section discusses in detail the various syndromes associated with chromosomal abnormalities before describing more general matters. These include the fascinating and thought-provoking relationships between fingerprints, mental abnormality and extra chromosomes, and the problem of the relation between chromosomal abnormalities and acquired diseases like cancer.

Anybody writing a textbook in a rapidly developing field must expect to be overtaken by events, but it is particularly unfortunate that the full impact of the fluorescent and Giemsa banding techniques on the identification of human chromosomes has come after most of this book was written. These latter methods are certainly discussed but the largely redundant and inadequate earlier methods of identification are still given equal weight. More seriously many of the ambiguities of chromosome assignment which appear in the later sections could have been removed.

The outline of molecular genetics, which authors of this kind of text nowadays rightly feel they must include, serves at least one useful purpose in that it underlines the complete inadequacy of current thinking on the control of the expression of genes in explaining the necessity for dosage compensation in the X or the far-reaching effects of any particular trisomy. Neither the bacterial repressor mechanism nor even the hypotheses involving chromatin condensation in gene switching lead one to predict that an extra complete chromosome should have such disastrous effects. The problem is challenging; obviously tolerance of simple heterozygosity is essential but does this include control sequences as well as sequences specifying proteins? Tandem doubling of particular loci must also be allowed; otherwise one cannot account for the increase of genetic material on the evolutionary time scale or for the evolution of haemoglobin. In addition the new methods of identification are showing that translocations of visible portions of chromosomes are common, which suggests that the functional relations of large sections at least of chromosomes are not essential. The basic requirements of life and even the simple control of the presence of enzymes are, however, compatible with the wide karyotypic variation common in tissue culture lines. It seems inescapable that it is only the ordered develop-

ment of a differentiated organism which requires a precise chromosome complement, but how are these chromosomal interactions mediated?

A book like this seems therefore to be essential reading for anybody concerned with gene expression and with the investigation of human chromosomal abnormality and genetic counselling.

P. M. B. WALKER

Variable structure

Theory and Applications of Variable Structure Systems. Edited by R. R. Mohler and A. Ruberti. Pp. x+232. (Academic: New York and London, December 1972.) \$8.

"VARIABLE structure" in the title is not closely defined anywhere in the book, but has been interpreted by the different authors to include any system which cannot be described by linear differential (or difference) equations with constant coefficients. This is not an easily defensible terminology, since the structure of systems which cannot be described in this simple way is usually no less fixed than the structure of those which can. Indeed, the most interesting results presented by the authors relate to fixed aspects of structure.

Nevertheless, the motivating idea of the seminar from which the papers are taken is clear. Much work has been done on linear constant systems, and many of their structural properties are known. More general systems can have different structural properties, which may be better adapted to describing a given physical situation. The aim of the seminar was to bring together applied mathematicians interested in generating and applying results for such systems.

The outcome is an interesting collection of papers which give a view of the present state of the subject. As would be expected, most progress has been made where the problems could be most closely restricted. In particular the theory of bilinear systems has now emerged as an incomplete but already well developed area. The work of Ruberti and of Brockett on the theory of these systems is particularly noteworthy, while Mohler lists a wide range of actual or potential applications: these include economic, biological and engineering systems.

Other topics include identification, modelling, model approximation and near-optimal control. Several papers deal with biological or medical applications—models of the brain, prostheses, ecological systems—where linear constant models have limited use. The book will interest a wide range of readers, but much of its content is inaccessible without a good mathematical background. H. H. ROSENBROCK

De Vitae Origine

The Origins of Life on Earth. By L. E. Orgel. Pp. 237. (Chapman and Hall: London, June 1973.) £3 boards; £1.95 paper.

Origin and Development of Living Systems. By J. Brooks and G. Shaw. Pp. xi+412. (Academic: London and New York, May 1973.) £5.

THE arrival of the space age has rocketed the interest in the study of the origin of life to new heights. The search for life beyond the Earth, the declared goal of space biology, is intimately associated with the scientifically larger question of the origin of life in the Universe. The Oparin-Haldane hypothesis of chemical evolution has now become the focus of extensive and sophisticated investigations in many laboratories around the world.

The awareness of the general public and the interest evinced by the non-specialist in science has created a demand for the latest information on the subject in what augurs to be a flood of publications on the beginnings of life. Orgel has written, not for the professional biologist or chemist, but for the freshman in college, the advanced high school student and the general reader, who has a limited knowledge of chemistry and molecular biology. Orgel himself is one of the leading practitioners in the chemistry of the origin of life. A theoretical chemist, who has turned to biochemistry and organic chemistry, he has successfully elucidated a number of chemical pathways presumed to be necessary prerequisites for the emergence of life.

From a discussion of the historical background he gives an account of the history of the Earth's primitive atmosphere and the sources of energy necessary for the synthesis of organic compounds. He then describes the processes which gave rise to the building blocks of life. Now comes the opportune moment to examine our concept of life, before embarking on a search for life beyond the Earth.

I was disappointed that a good portion of the book has been spent on the background necessary for understanding the problem. I missed the excitement and thrill which I would have expected in picking up a book on the origin of life, written by a scientist actively engaged in its study. But the clarity of presentation, and the thoroughness of the discussion on the critical issues, make this book a valuable addition to the shelves of the general reader interested in an up-to-date and succinct account of current thinking on the problem of life's beginning.

Brooks and Shaw, on the other hand, have approached their study of the origin of life from a very different point of view. Having been involved in the

study of pollen grains and their relationship to microspores, they became interested in the ancient Precambrian sediments. These studies led them to the analysis of some of the oldest sediments on Earth. According to their own admission, they felt that there was a great lack of knowledge among chemists, both in the organic and inorganic fields, about the relationship of the geochemical aspect to the problem of the origin of life. Brooks and Shaw present the geochemical background to chemical evolution in readable fashion.

The book starts with an account of the Universe and the evolution of the Earth and its atmosphere. The authors then proceed to the chemicals of life, and finally to an analysis of the Precambrian fossil record and the story of carbonaceous chondrites. Although this book is designed for the non-specialist and the general reader, a great deal of the material would be of value to the investigator and advanced student.

Of major interest are the chapters dealing with the Precambrian fossil record. Here I was delighted with the note of personal involvement. The excellent pictures provide a refreshing dimension to a stimulating discussion.

The weakness of the book is that the authors, on the one hand, have attempted to cover far too much within a limited number of pages, and, on the other, aimed at too large an audience. If the book was intended for the non-specialist, some of the chapters, though well written, may be above his grasp.

CYRIL PONNAMPERUMA

Early quanta

Quantum Mechanics: New Approaches to Selected Topics. By H. J. Lipkin. Pp. xv+465. (North Holland: Amsterdam and London; American Elsevier: New York, 1973.) Dfl. 95; \$33.30.

THIS book grew from the lecture notes accrued by the author while giving postgraduate courses on quantum mechanics over a decade both in the United States and in Israel. It differs considerably from many of the standard texts intended for use with such courses. It comprises six monographs on selected topics such as the Mössbauer effect, many body quantum mechanics, Kaon physics, scattering theory, Feynman diagrams and relativistic quantum mechanics. These are presented at a much earlier level than in the conventional texts alongside which they are intended to be read.

The first is based on Dirac's use of the concept of photon polarisation as a general introduction to quantum

mechanics. The idea of states of polarised light leads on to the development of a matrix representation, essentially Jones's calculus. There are other texts such as *States, Waves and Photons* by Simmons and Guttman which are both more readable and comprehensive in their coverage of this topic. Some reference by the author to Jones and Mueller matrices might be useful as reference.

The second monograph, comprising three chapters, deals with momentum transfer with specific reference to the Mössbauer effect. Here a clear and concise listing is given which includes details of earlier experiments on resonance phenomena. Momentum transfer within a solid matrix, involving phonon interactions and nuclei, is then described with emphasis on beta decay and electron scattering by complex nuclei. This monograph is mainly concerned with the concept of a form factor and requires only an understanding of a free particle and harmonic oscillator in order to be led up to a formal treatment of second quantisation.

The treatment of many particle quantum mechanics follows in the next monograph which deals in turn with fermions and the application of fermion pairs to superconductivity. The concepts of time-dependent perturbation theory are then introduced in the next monograph on the treatment of Kaon decay. A chapter on one-dimensional scattering models provides an excellent introduction to this important part of quantum mechanics and is followed by three chapters dealing with the many body interaction problem. A comprehensive presentation of Thomas-Fermi and Hartree-Fock models is followed by a treatment of elementary excitation processes including giant resonances. The final section in this monograph introduces the reader to spectral and Green's function without entering fully into their applications.

The reader is introduced next to time-dependent perturbation theory through Feynman diagrams, using third order 'bremsstrahlung' processes as an example. The polaron is also used as an example of a quantum field approach to solid state physics.

The final monograph introduces the reader to symmetries, invariance and relativistic quantum mechanics. Starting with the Dirac equation, the reader is introduced to gauge transformations, the Lorentz group as a case of symmetry transformation and finally to charge conjugation.

Each monograph in this excellent book is introduced by a summary and ends with a problem test. The book is recommended to graduate students and research physicists looking for a pedestrian but fairly informal treatment of these topics. D. W. GOODWIN

Chromosomes decide sex

Genetics of Sex Differentiation. By Ursula Mittwoch. Pp. xi+253. (Academic (Harcourt Brace Jovanovich): New York and London, June 1973.) \$16.50.

THAT sex was another example of the transmission of hereditary factors rather than an environmentally controlled duality was probably first postulated by Mendel but was still a pertinent question at the turn of the century. In the companion book, *Sex Chromosomes* (Academic, London, 1967), Ursula Mittwoch briefly reviewed the early concepts of how sex is determined, which followed the discovery of the sex chromosomes. She dealt in detail with the mechanism in plants and animals, presenting the view that sex determination may be a process not depending on specific differences of protein production.

The present book is a general and useful introduction to the subject of sex development and its control especially in vertebrates and amplifies this concept. It is important, at this stage, to distinguish between sex determination (the primary event that decides on the type of gonad that an embryo makes) and sex differentiation (the subsequent effects of the gonad on embryogenesis of the other sex structures). The aim of the book is to give substance to the idea that sex determination results from an epigenetic control that primarily resides in whole chromosomes or chromosome segments. These, because they are visibly different, are assumed to have an effect on development "which cannot be accounted for in terms of genes". In other words the suggestion is that sex chromosomes determine sex by functioning as larger units rather than through the action of individual genes: this is the first part of the hypothesis. For its second part the author turns to non-genetic DNA, its redundancy, variation and function and to the view that sex chromosomes may control sex determination by affecting differentially the rates at which cells divide and grow during embryogenesis.

The thesis that Mittwoch presents is contrasted with other hypotheses involving discrete genic determinants, as the mainspring for gonadal differentiation, and she marshals various lines of support for it. Examples are taken from the effect of heterochromatic B chromosomes on mitotic rates in plants, the effect of X chromosomes on the mitotic cycle of human cells *in vitro*, the effect of growth rates on embryonic differentiation, particularly the analysis of transdetermination of *Drosophila* imaginal disks, and finally the effect of the various environmental influences on embryonic differentiation. Indeed, the bulk of the first six of the seven chapters

of the book is an introduction to the thesis developed in the final chapter.

Irrespective of whether the hypothesis which this book discusses will ultimately turn out to be right or wrong, it is important that attention has been drawn to it. Experiments of nature should be scrutinised and experiments should be devised to see if they falsify the idea, but until then it should rank with the discrete gene hypotheses of sex determination.

PAUL E. POLANI

Insects of Europe

A Field Guide to the Insects of Britain and Northern Europe. By Michael Chinery. Pp. 352. 60 plates. (Collins: London, November 1973.) £2.95.

THIS latest addition to the Collins' Field Guide series follows their recent pattern of including Europe, in this case Europe north of the Alps. But anyone expecting to identify any insect encountered, either in this country or while holidaying on the Continent, will be sadly disappointed. This field guide only claims to enable people with no previous knowledge of the subject to first make sure that what they have found is an insect, and second to place it in the correct order and either the appropriate superfamily or family. For further identification to species a fairly extensive bibliography is provided, although some may be recognised from the superb colour plates. Those depicting lepidopterous larvae are, however, rather garish and are irritatingly interleaved with the glossary.

The glossary surprisingly omits the only obvious competitor, *The Oxford Book of Insects*. Although this new guide does not contain the comprehensive coverage of butterflies and dragonflies, it has advantages over the other in containing simplified keys, well illustrated with thumbnail sketches. The author admits that these ignore the 'odd-man-out' but they are nonetheless very valuable.

There is an excellent general introduction to entomology containing essential details of anatomy and biology, with notes on collecting and preserving insects. Unfortunately the Code for Insect Collecting is printed on the dust jacket which may become separated from the book. There is a concise chapter on classification and taxonomy and a list of entomological suppliers. It is unfortunate that the Lepidoptera section of the revised Kloet and Hincks could not be used, but the use of archaic Psocoptera nomenclature is less forgivable. Little content would have been lost by covering only British insects, although I believe there is a similar need for such a guide on the Continent. The book will sell on first impressions alone, and is extremely good value.

R. COLIN WELCH

Theory of athletics

The Mechanics of Athletics. By G. Dyson. Pp. 240. (University of London: London, September 1973.) Boards £3.30; paper £2.20.

THIS book, by the Director of Physical Education at Winchester, formerly Chief National Coach to the Amateur Athletic Association, is one that every athlete wishing to add to his skill should continually study. Mr Dyson has vast experience of athletics and coaching, and makes clear at the outset his creed that the theoretical understanding of what one is supposed to be doing is the key to improvement. "Those enamoured of practice without science [theory] are like a pilot without rudder or compass, never certain where he is going", he quotes (from Leonardo da Vinci). But he well understands that the theory must be allowed to sink in and become so much part and parcel of the method of the performer that he comes to do the right things automatically, and it is the task of a coach to induct such theory with skilful application. This of course holds for most if not all instruction: the existing level of a pupil's state of understanding and attainment must be assessed and the philosophic pill gilded accordingly.

After first lucidly expounding what is needed to be known for his purposes about motion and force, and about rotatory motion with its less obvious centripetal and gyroscopic consequences, Mr Dyson takes his readers through everything that he has found to be essential in comprehending the correct principles of running, hurdling, jumping (high and long), pole vaulting, shot putting, and throwing the hammer, discus, and javelin. No great prior knowledge of mechanics is required from the reader. Any athlete who has come to a steady state of achievement, yet nevertheless feels he ought to be able to do better, may well find the secret to his making fresh progress in the relevant chapters of this book. It has seen five previous editions, been translated into several languages including Japanese (who mean business in the sporting world, be it noted), and not surprisingly is required reading in many universities.

It is hard to know how many readers of *Nature* may also be interested in athletics, but if they are not themselves, their children or friends may be, and whether or not these have access to coaching at high level, this book could well make an admirable scientific gift of permanent value that would do much to add to their enjoyment of athletics both through greater appreciation of the fundamentals and improvement of their own performance.

R. A. LYTTLETON

matters arising

30 S DNA: intermediate or artefact?

SIR,—Goldstein and Rutman¹ have pulse-labelled the DNA of Ehrlich ascites cells growing intraperitoneally in a mouse with either ³H or ¹⁴C-thymidine. The DNA was subsequently extracted from the cells and its molecular weight measured on gradients of alkaline sucrose.

After short pulse times (<2 min), the labelled DNA had a sedimentation coefficient of 9S indicating the presence of short intermediates in DNA replication ("Okazaki" fragments²). After longer times (10–30 min) they observed labelled DNA which sedimented somewhat more slowly than the bulk DNA (30S as opposed to 44S) and they claimed that this represented a discrete intermediate in the replication of DNA. This interpretation is improbable.

If a long molecule is labelled at one end (as happens with a short pulse label) and then sheared, the labelled molecules will always appear to have lower molecular weights than the bulk material. This artefact is a result of the different labelling patterns of pieces that derive from molecules labelled only at the ends, as compared with molecules labelled throughout their lengths. This effect has been described qualitatively in ref. 3 and a complete mathematical analysis can be found in ref. 4.

The observation of apparently slightly smaller lengths of pulse-labelled DNA (for example, 30S as opposed to 44S) is therefore not in itself evidence for a special class of DNA molecules. It merely demonstrates that the label goes on to the ends of growing DNA strands. This criticism does not apply to the observation of 9S DNA. The most plausible interpretation of the data is that there is an intermediate in DNA replication of about 0.4×10^6 daltons and that this is linked directly on to the end of the growing DNA molecule.

Yours faithfully,

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Drs Goldstein and Rutman respond: The points from our study¹ of 'self-chase' Ehrlich ascites tumours which supported the DNA replication model involving sequential tandem ligations of 9S→30S and 30S→44S were: (1) maximal radioactive pulse-labelling of new 9S DNA by 2 min; (2) sharp peak transitions in alkaline sucrose gradients of labelled DNAs from alkaline-lysed cells, with no broad envelope of DNA sizes observed (2–120 min); (3) time of appearance of bulk-size labelled 44S DNA, 20–30 min, was best explained by ligation of 20 of the 9S chains to form 30S DNA and further ligation of 3 of 30S to form 44S; if 9S were directly linked to the end of a growing, long molecule, as proposed by Lehmann and Ormerod^{3,4}, it should have taken 120 min to form 44S with an initial polymerisation rate of 600 deoxynucleotides per min; (4) at that rate of synthesis, excess DNA per cell would have been made in a 10 h S phase¹.

If ligation processes are under the control of replication fork(s)¹, before any ligation could begin, RNA primer molecules, to which Ehrlich cell Okazaki fragments are probably covalently linked^{5,6}, would have to be removed and replaced by DNA, as described for *E. coli*⁷. These latter mechanistic requirements with respect to 9S DNA taken in conjunction with our approximate kinetic analyses of 44S formation indicate that, like 9S, 30S DNA is a discrete replicative intermediate and not an artefact. The crux of this discussion rests on our choice between alternative interpretations of the data.

We have subsequently shown that bifunctional nitrogen mustard interferes with both synthesis of 9S DNA chains and their eventual step-wise conversion to 44S through 30S DNA⁸.

Yours faithfully,

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¹ Goldstein, N. O., and Rutman, R. J., *Nature new Biol.*, **244**, 267 (1973).

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Impact erosion by jets of dilute polymer solutions

SIR—Results described in the letter by Kudin *et al.*¹ increase the number of types of materials which have been cut by an impacting jet containing traces of soluble polymers. The hypothesis propounded by the authors, that increase in erosion capability is due to the rigidity of large aggregates of the polymer when subjected to high loading rates is not, however, correct.

In work carried out at the Universities of Leeds and Missouri-Rolla^{2,3} work on polymer addition has shown an improvement over plain water jets in impact erosion of rock (Fig. 1). The fluid was filtered at 10 μ m before entering the pumping system. This would hold back any large aggregates which Kudin *et al.*, suggested act as solid particles at short time scales and cause the

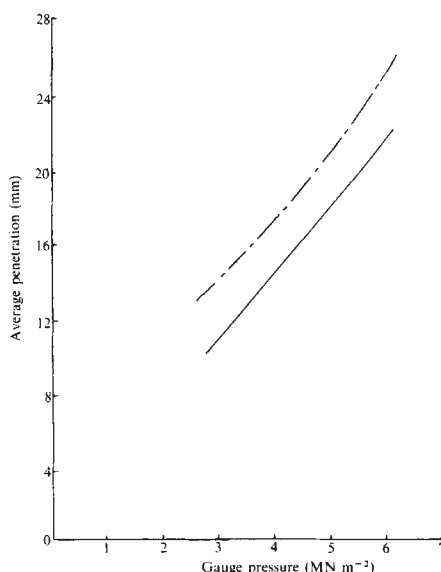


FIG. 1 Comparison between penetration of a water jet and jet containing Polyox into sandstone under varying pressure (after ref. 2). —, Ordinary jet; ---, with 0.1% Polyox.

erosion. The likely cause of the improvement is the increased cohesion of the jet stream, rather than any change in physical property of the fluid—such changes in density and viscosity are negligibly small in the concentrations used—or solid particle behaviour of polymer and associated solvent. That this is so is shown by the increasing effect of the polymer on jet cutting ability as the target is moved away from the nozzle (Fig. 2). Photographic evidence of the improvement in cohesion is available⁴ for

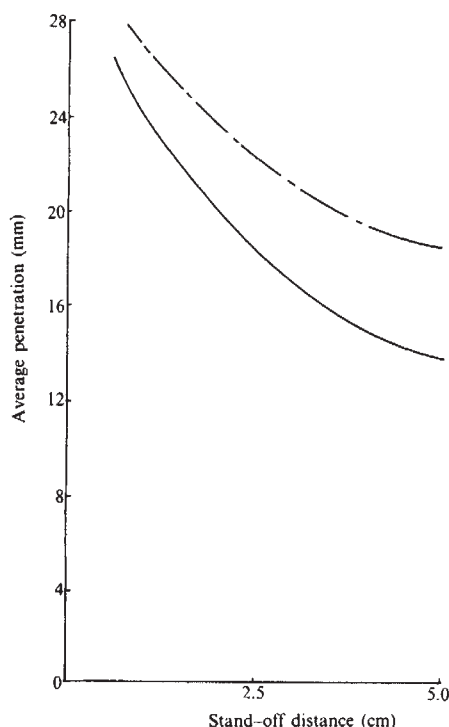


FIG. 2 Comparison between penetration of a water jet and a jet containing Polyox into sandstone with increase in target distance (after ref. 2). —, Ordinary jet; ---, with 0.1% Polyox.

studies where the surrounding fluid is air and this improvement may be enhanced when the jets are submerged as apparently was the case in ref. 1. The presence of the central core on the surface of the target during the erosion process (Fig. 2c of ref. 1) is a further indication that the impacting fluid, rather than a series of solid impacts as suggested¹ is the cutting agent since such a cone is a feature of water jet erosion⁵.

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The individual and the information problem

THE skewness of the distribution of journals in which individual scientists and research institutes as a whole publish their findings, shown by Blaxter and Blaxter¹ in their recent paper, will come as no surprise to most research librarians. A similar phenomenon of the distribution of journals relevant to particular specialities has been known for a number of years.

As long ago as 1927, Gross and Gross², studying chemical literature, developed a theory of evaluating periodicals based primarily on the assumption that the value of a periodical to a scientific worker is in direct proportion to the number of times it is cited in the scientific literature. The results obtained showed this typical distribution, with a relatively small number of periodicals containing a high percentage of the papers thought to be relevant to the subject, but with the subject's total literature widely dispersed through many journals. A similar conclusion was arrived at by Bradford³ and later by others working on Bradford's Law^{4,5}. Over the past 25 yr the literatures of many different subject fields have been analysed, and all show similar distributions⁶⁻¹⁰. Garfield¹¹ has recently shown this same skew distribution to be a property of the scientific literature as a whole, important work being concentrated in relatively few journals.

Blaxter and Blaxter's estimations for the optimum size of a working library satisfying 90% of the needs of 90% of its users could probably be reduced even more, as it is very likely that several of the twenty-five journals relevant to each of the five subject fields would be the same. The 25 yr estimated working life of the library stock only holds true if the current research interests of the workers do not change appreciably during this time. When major new fields are entered new journals are needed. Similarly, journals supporting superceded research work should be pruned from the library stock long before they are 25 yr old.

All librarians are very well aware of the costs of subscribing to, and maintaining long back-files of journals, and much bibliometric work has been carried out in an attempt to minimise these costs. This indication that users appreciate these points too makes a welcome

alternative to their more usual requests for yet more new journals.

Yours faithfully,
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Research Library,
The Boots Company Ltd,
Nottingham, NG2 3AA

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not included in the Monthly Books Supplement

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[1012] Higher Education and Scientific Research—Report of a Study Group. (British Association Publication 73/1.) (London: British Association for the Advancement of Science, 1973.) 30p post free.

[1112] The Measurement of Frequency and Time Interval. By Dr L. Essen. (National Physical Laboratory.) Pp. v + 55. (London: HMSO, 1973.) £1 net.

[1312] *Annals of Human Biology*, Vol. 1, No. 1, January 1974. Edited by O. G. Edholm, G. A. Harrison and J. M. Tanner. Pp. 1-136. (Journal of the Society for the Study of Human Biology.) Published quarterly. Annual Subscription price (payable in advance) £12, postage paid. Subscribers in the USA, Canada and Mexico (air-freight) £13.50; \$33.75 postage paid. (London: Taylor and Francis, Ltd., 1974.)

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[1122]